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Cytotoxic evaluation of some plants against renal cancer cell lines

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ABSTRACT: In the current study, we evaluated the cytotoxic activities of 21 extracts from 7 plants belonging to 4 families. The dichloromethane, ethyl acetate, and methanol extracts of the plants were screened for their cytotoxic activities on the renal (A498 and U031) cancer cell lines. 8 extracts exhibited more than 50% growth inhibition at a 25 ug/mL concentration on both renal cancer A498 and UO31 cell lines. The highest cytotoxic activity on the renal cancer UO31 cell line was observed for the methanol extract of the aerial parts of *Telekia speciosa* with 66% inhibition at a 25 ug/mL concentration. The highest cytotoxic activity on the renal cancer A498 cell line was found for the dichloromethane extract of the aerial parts of *Coronilla varia* with 64% inhibition at a 25 ug/mL concentration.

KEYWORDS: Cytotoxic activity; renal cancer; *Telekia speciosa*; *Coronilla varia*.

1. INTRODUCTION

Cancer remains the second leading cause of death followed by cardiovascular disease in globe. It is defined by uncontrolled cell division and its metastatic properties [1]. The second most common cancer of the urinary system is kidney cancer [2]. In 2020, 431,288 new instances and approximately 179,000 deaths of renal cancer were reported on a worldwide scale [3]. Kidney cancer has been reported to be the ninth most common cancer among men and the fourteenth most common among women. The majority (90%) of the kidney cancer cases consist renal cell carcinoma (RCC), 70% clear cell RCC, 10-15% papillary RCC, 5% chromophobe RCC and ≤1% other subtypes [4]. Older age, male sex, smoking, hypertension, and obesity were explained as the risk factors for RCC [5]. Renal cell carcinoma (RCC) is characterized by a marked metastatic potential and is the third most common cancer to metastasize to the head and neck, following breast and lung cancer [6]. Numerous studies have been carried to discover new effective compounds from natural sources for various cancer types [7,8]. Previous studies have shown that plant-derived natural compounds such as glycosides, alkaloids, tannins, terpenes, coumarins, and flavonoids and plant extracts have cytotoxic potential against cancer cells such as renal, lung, breast, and colorectal cancer cells [9-19].

The aim of present study is to evaluate the cytotoxic activities of seven plants distributed four families growing in Turkey, against the renal (A498 and U031) cancer cell lines. Additionally, this research introduces new plant sources whose cytotoxic activities have not been reported previously.

2. RESULTS AND DISCUSSION

Seven plants were screened against the renal (A498 and U031) cancer cell lines for the first time in this study.

Cytotoxic activities of twenty-one extracts obtained from seven plants tested against A498 and U031 renal cancer cell lines are shown in Table.

Among twenty-one extracts screened, thirteen extracts (61.9%) showed growth inhibition of more than 50% at a 25 ug/mL concentration in both renal cancer (A498 and UO31) cell lines. The most potent activity was found for the methanol (MeOH) extract of *Telekia speciosa* (Schreb.) Baumg. exhibited against UO31 cell line with 66% inhibition at a 25 ug/mL concentration. The dichloromethane (DCM) extracts of *Salvia forskaehlei* L. and *Thymus nummularius* M.Bieb. and the MeOH extract of *Coronilla varia* L. were also

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active with the inhibition of more than 60% at a 25 ug/mL concentration against UO31 cell line. The DCM extracts of *Anthemis tinctoria* L. var. *tinctoria* and *Coronilla varia* and the MeOH extract of *Telekia speciosa* were found the most active extracts against A498 cell line with the inhibition of more than 60% at a 25 ug/mL concentration. Of all seven DCM extracts, five showed more than 50% growth inhibition at a 25 ug/mL concentration in both renal cancer cell lines. Five MeOH extracts and three ethyl acetate (EtOAc) extracts inhibited the growth of both renal cancer cell lines by more than 50% at a 25 ug/mL concentration. All three extracts of *Thymus nummularius* and *Cryptomeria japonica* (Thunb. ex L.f.) D. Don showed more than 50% growth inhibition at a 25 ug/mL concentration in both renal cancer cell lines.

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

				Inhibition %					
				A498			UO31		
Plants	Parts	Voucher No	Collection sites	1	2	3	1	2	3
Asteraceae									
Anthemis tinctoria L. var. *pallida* DC.	A	IMEF1180	Giresun-Şebinkarahisar	40	45	**54**	43	**53**	**53**
Anthemis tinctoria L. var. *tinctoria* L.	A	IMEF1181	Trabzon-Tonya	**63**	41	46	**56**	43	**54**
Telekia speciosa (Schreb.) Baumg.	A	IMEF1182	Trabzon-Altındere	**53**	49	**62**	46	49	**66**
Lamiaceae									
Salvia forskaehlei L.	A	IMEF1190	Trabzon-Tonya	**55**	37	**53**	**61**	45	**64**
Thymus nummularius M.Bieb.	A	IMEF1192	Trabzon-Zigana pass	**56**	**59**	**56**	**62**	**54**	**52**
Leguminosae									
Coronilla varia L.	A	IMEF1199	Van-Campus of the Univ.	**64**	**50**	48	**59**	**52**	**62**
Taxodiaceae									
Cryptomeria japonica (Thunb. ex L.f.) D.Don	L	IMEF1189	Cultivated-Trabzon	**56**	**57**	**50**	**56**	**55**	**59**

1. DCM extract.
2. EtOAc extract.
3. MeOH extract.
A. Aerial parts.
L. Leaves.

Previously four of these seven plants were tested on different cancer cell lines. For example, the ethanolic extracts of *Coronilla varia* were tested against the HeLa (human cervical adenocarcinoma) [20] and MCF-7 cancer cell lines [21]. EtOAc and MeOH extracts of *C. varia* were reported the most effective extracts on MDA-MB-231 and MCF-7 breast cancer cell lines and to contain flavonoids, polyphenolic compounds and coumarins [22]. Cryptoquinone obtained from the *n*-hexane extract of *Cryptomeria japonica* barks has been reported strongly cytotoxic against P388 (mouse lymphoid neoplasm) cells [23]. Ctyptotrione obtained from the MeOH extract of *Cryptomeria japonica* barks has been shown medium cytotoxic activity against human oral epidermoid carcinoma KB cells [24]. Cytotoxic activities of terpenic compounds obtained from the barks of *Cryptomeria japonica* were evaluated against HL-60 and HCT-15 cell lines [25]. The leaf extracts of *C. japonica* and ferruginol has been reported to have cytotoxic activities against A549 and CL1-5 human lung adenocarcinoma cells [26]. The extracts of *Anthemis tinctoria* L. var. *tinctoria* were found to be cytotoxic on the rat glioma C6 cell line and to contain phenolic compounds as chlorogenic acid, gentisic acid and apigenin-7 glycoside [27]. The methanolic extract of *Anthemis tinctoria* exhibited no effect on the KB (oral epidermoid carcinoma) and LNCaP (prostate carcinoma) cells [28]. Cytotoxic activities of the terpenoids obtained from *Telekia speciosa* were examined on prostate carcinoma cells (PC3 and Du145), human skin fibroblasts and melanoma (A375, WM793 and Hs294T) cell lines [29]. Cytotoxic activities of the essential oils from the flowers, leaves and roots of *Telekia speciosa* were evaluated on A375 melanoma cells [30].

No reports in the literature dealing with the cytotoxic activities of *Anthemis tinctoria* L. var. *pallida*, *Salvia forskaehlei* and *Thymus nummularius* (Syn.*Thymus pseudopulegioids*).

In the current study, the DCM extracts of *Anthemis tinctoria* L. var. *tinctoria* and *Coronilla varia* and the MeOH extract of *Telekia speciosa* were found the most active extracts against A498 cell line with the inhibition of more than 60% at a 25 ug/mL concentration. The DCM extracts of *Salvia forskaehlei* and *Thymus nummularius* and the MeOH extract of *Telekia speciosa, Salvia forskaehlei* and *Coronilla varia* exhibited more than 60% growth inhibition at a 25 ug/mL concentration against UO31 cell line. The remaining extracts of the studied plants showed between 37% and 60% growth inhibition at a 25 ug/mL concentration in both renal cancer (A498 and UO31) cell lines.

3. CONCLUSION

This study is the first cytotoxicity screening performed on these seven plants against the renal (A498 and U031) cancer cell lines. Three of these seven plants (*Anthemis tinctoria* L. var. *pallida*, *Salvia forskaehlei* and *Thymus nummularius*) were tested for the cytotoxic activities in this study for the first time.

Previous studies on the among the plants exhibited more than 60% growth inhibition at a 25 ug/mL concentration against renal cell lines (*Anthemis tinctoria* L. var. *tinctoria, Coronilla varia, Salvia forskaehlei, Thymus nummularius* and *Telekia speciosa*) *Anthemis tinctoria* L. var. *tinctoria* has been reported to contain fifteen flavonoid aglycones, twelve glycosides, and one caffeoyl-*O*-flavonoid [28]. *Coronilla varia* has been explained to contain flavonoids, polyphenolic compounds and coumarins [22]. Previously, the ethanolic extracts of leaf and flower of *Thymus pseudopulegioides* have been studied for antioxidant and antibacterial activities and the total phenolic contents. The leaf extract has been reported to show better antioxidant activity than flower extract [31]. In another study the antimicrobial, antiviral and antioxidant activities on the essential oil and extracts of *T. pseudopulegioides* have been reported [32]. The triterpenes, sesquiterpene lactones, carotenoids, ferulic and caffeic acid derivatives, flavonoids obtained from the extracts of *Telekia speciosa* have been explained in the previous studies [29,33]. Previous phytochemical studies on the *Salvia* species have shown the presence of monoterpenes, diterpenoids, triterpenoids, flavonoids and phenolic acids [34-36].

There are no reports in the literature dealing with the cytotoxic activities of the three plants (*Anthemis tinctoria* L. var. *pallida*, *Salvia forskaehlei* and *Thymus nummularius*) and with the phytochemical contents of *Salvia forskaehlei*.

In conclusion, bioactivity-guided fractionation of the *Anthemis tinctoria* L. var. *tinctoria, Coronilla varia, Salvia forskaehlei, Telekia speciosa* and *Thymus nummularius* 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. Voucher specimens are deposited in the Herbarium of School of Pharmacy of Istanbul Medipol University, Istanbul, Turkey (IMEF). Used plant part is leaves for *Cryptomeria japonica*. Aerial parts are used for the remaining six plants.

4.2. Preparation of extracts

The leaves/aerial parts of the plants were dried and coarsely powdered and then sequentially extracted with DCM, EtOAc, and MeOH at room temperature. The extracts were separately concentrated in a rotary evaporator under reduced pressure to dryness. All extracts were stored at −20^{0}C prior to screening.

4.3. Cytotoxic activity assay

The assay used for this study was a two-day, two cell line XTT bioassay [19], 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 [18]. Cytotoxic activities of extracts are shown in Table.

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