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ORIGINAL ARTICLE / ÖZGÜN MAKALE

CYTOTOXICITY SCREENING AND ANTIOXIDANT CAPACITY ASSESSMENT OF THE INNER PERIANTH SEGMENTS OF 14 RUMEX SPECIES GROWN IN TÜRKİYE

TÜRKİYE'DE YETİŞTİRİLEN 14 RUMEX TÜRÜNÜN İÇ PERİANT SEGMENTLERİNİN SİTOTOKSİSİTE TARAMA VE ANTİOKSİDAN KAPASİTE DEĞERLENDİRMESİ

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ABSTRACT

Objective: *Breast cancer is one of the most prevalent cancer types worldwide. Antioxidant sources may prevent the occurrence of cancer. Natural sources rich in phenolics, thus, may provide alternate agents in the management of breast cancer. Rumex species are widely distributed in Turkish flora. Emerging evidence has pointed out the antitumoral property of Rumex species on a variety of cancer cells. In the present study, we propose to test the ethanolic extracts of the inner perianth segments of 14 Rumex species on four breast cancer cells with different origins. We also demonstrated their toxicity on healthy cells.*
Material and Method: *We performed the resazurin reduction assay to examine the cytotoxicity and toxicity. Furthermore, we determined the phenolic contents of the extracts as an indicator of their antioxidant profile and ascertained their antioxidant activities by DPPH radical, ABTS radical cation scavenging activity and cupric ion-reducing antioxidant capacity assays.*
Result and Discussion: *The ethanolic extracts of the inner perianth segments of Rumex species exhibited remarkable cytotoxicity profiles neither on breast cancer cells nor on healthy H9c2 rat myoblastoma cells. However, they usually displayed strong antioxidant activities due to possessing high phenolic content.*
Keywords: *Antioxidant, breast cancer, cytotoxicity, rumex, total phenol*

ÖZ

Amaç: *Meme kanseri dünya çapında en yaygın görülen kanser türlerinden biridir. Antioksidan kaynaklar kanserin oluşumunu önleyebilir. Dolayısıyla fenolikler açısından zengin doğal kaynaklar meme kanseri tedavisinde alternatif ajanlar sağlayabilir. Rumex türleri Türkiye florasında geniş bir dağılım göstermektedir. Bilimsel çalışmalar Rumex türlerinin çeşitli kanser hücreleri üzerindeki antitümöral özelliğine işaret etmektedir. Bu çalışmada, 14 Rumex türünün iç periant segmentlerinin etanolik ekstrelerinin farklı kökenlere sahip dört meme kanseri hücresi üzerinde test edilmeleri amaçlanmıştır. Ayrıca sağlıklı hücreler üzerindeki toksisiteleri de değerlendirilmiştir.*
Gereç ve Yöntem: *Sitotoksisite ve toksisiteyi incelemek için resazurin redüksiyon yöntemi kullanılmıştır. Ayrıca; antioksidan profillerinin bir göstergesi olarak ekstrelerin fenolik içerikleri belirlenmiş ve DPPH, ABTS radikali süpürücü aktivite ve bakır iyonu redükleyici antioksidan kapasite yöntemleri ile antioksidan karakteristikleri belirlenmiştir.*

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Sonuç ve Tartışma: *Rumex türlerinin iç periant segmentlerinin etanolik ekstreleri, sağlıklı H9c2 sıçan miyoblastoma hücreleri üzerinde toksik olmamasına rağmen, meme kanseri hücreleri üzerinde dikkate değer sitotoksisite profilleri sergilememiştir. Ancak; genellikle yüksek fenolik içeriğe sahip güçlü antioksidan aktiviteler sergilemektedirler.*
Anahtar Kelimeler: *Antioksidan, meme kanseri, rumex, sitotoksisite, total fenol*

INTRODUCTION

Rumex L. (Polygonaceae family) comprises more than 200 species and is widely distributed worldwide, in the northern hemisphere in particular. The genus mostly consists of perennial herbs with strong roots, paniculate inflorescences, and triangular fruits that are coated in the ampliate inner perianth [1,2]. Scientific literature has pointed out that the plants involved in the genus *Rumex* have been used either traditionally as edible plants or for curing several diseases worldwide [1-6]. Emerging data has further revealed the pharmacological activities of *Rumex* extracts or their containing compounds in preclinical experiments. The studies focusing on the determination of the phytochemical composition of *Rumex* species indicated the presence of hundreds of phytochemicals from different chemical classes including anthraquinones, flavonoids, alkaloids, lignans, naphthalenes, stilbenes, tannins and terpenes [1,2].

The Turkish Plant Data Service (TÜBİVES), a biodiversity database of the plants in Türkiye, reported that 31 *Rumex* taxa exist in the flora of Türkiye [7-9]. To date, a number of *Rumex* species grown in Türkiye have been investigated by our group. We have contributed to the scientific knowledge in terms of their phytochemical or biological activity profiles [10-21].

Breast cancer is a life-threatening condition occurring in every country in the world. It is the most prevalent cancer type globally and is reported to cause 685 000 deaths in 2020. Surgery, radiation therapy, hormonal therapies, chemotherapy or targeted biological therapies are currently applied treatment options in breast cancer. Chemotherapy regimens are decided based on the cancer type (i.e., Cancers express estrogen receptor (ER) or progesterone receptor (PR), or overexpress the human epidermal growth factor receptor 2 (HER-2)/neu oncogene… etc.) [22]. Nature provides an indispensable source of new agents that can be used against numerous cancers. Newman and Cragg (2020) stated that only 29% of small molecule drugs approved between 1981 and 2019 were totally synthetic drugs while others are inclusive of natural products, their mimics or derivatives [23]. In the meantime, oxidative stress is involved in the occurrence of many diseases including cancer [24,25]. In fact, cancer initiation and progression have been associated with oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation [26]. Natural products are antioxidant sources due to possessing phenolics-rich phytochemical constituents [27]. Considering the association of cancer with oxidative stress, the investigation of cytotoxic and antioxidant profiles of natural products holds importance. In the 1920s, Essiac tea which is a blend of different herbs, including *Rumex acetosella* L. was promoted as a natural cancer treatment. This information indicates that *Rumex* species may have anticancer potential [28].

In the present study, we examined the cytotoxic profiles of the ethanolic inner perianth extracts of 14 *Rumex* species on breast cancer cells with different origins (i.e., they may include any of ER, PR, HER2/neu or not) and their toxicity profiles on healthy H9c2 rat myoblastoma cells. We further investigated the antioxidant properties of these extracts. Thus, we aim to provide a general overview about the potential of *Rumex* inner perianth extracts against breast cancer.

MATERIAL AND METHOD

Plant Materials

Aerial parts (including inner perianth segments, leaves, and roots) of 14 *Rumex* species, four of which are endemic to Türkiye, Polygonaceae, were collected from different locations of Türkiye in May to July 2018 and May to July 2019. The plants were identified by Prof. Dr. L. Ömür Demirezer and Dr. Pharm. Filiz Boyalı. Voucher specimens have been kept in the Herbarium of Hacettepe University, Faculty of Pharmacy, Ankara, Türkiye (HUEF) under associated HUEF codes. The detailed information

of collected plant samples is indicated below (Table 1).

Table 1. The location and altitude of the places where the investigated *Rumex* species were collected along with their herbarium numbers

Plant taxa	Collection date	Collection site	Altitudes	Herbarium number
Rumex alpinus L.	08.07.2019	Artvin	1100 m	HUEF 22051
Rumex amanus Rech.*	06.06.2019	Muğla-Marmaris Söğüt Village	0 m	HUEF 19043
Rumex caucasicus Rech.	31.05.2019	Uşak-İzmir Road	900m	HUEF 19046
Rumex chalepensis Mill.	02.06.2019	Uşak Kemalöz District	900 m	HUEF 19049
Rumex conglomeratus Murray	12.06.2018	Bursa İznik Gölü Area	85 m	HUEF 19036
Rumex crispus L	04.06.2019	Uşak Kemalöz District	900 m	HUEF 19045
Rumex cristatus DC.	31.05.2019	The area between İzmir-Kemalpaşa and Manisa-Turgutlu	68 m	HUEF 19050
Rumex grasilescens Rech.*	12.06.2018	The area between Bilecik and Kütahya, İnönü Junction	833 m	HUEF 19047
Rumex hydrolapathum Huds.	15.07.2019	Bolu Abant Lake Area	1350 m	HUEF 22057
Rumex sanguineus L.	06.06.2019	Muğla-Marmaris Söğüt Village	0m	HUEF 19051
Rumex tmoleus Boiss.*	13.05.2019	İzmir-Ödemiş, Bozdağ- Ovacık Village	1170 m	HUEF 19037
Rumex obtusifolius subsp. *subalpinus* L.	16.06.2019	Ankara Çayyolu Necatibey District	1042 m	HUEF 19086
Rumex olympicus Boiss.*	13.05.2018	İzmir-Ödemiş, Bozdağ	1190 m	HUEF 22052
Rumex pulcher L.	13.05.2018	İzmir-Ödemiş, Birgi	325 m	HUEF 19039

* Endemic plant

Cell Culture

The cell lines used in this study, their origins and maintenance conditions were previously reported [29]. Various breast cancer cells with different origins (MCF-7, MDA-MB-231, MDA-MB-468 and SKBR-3) and healthy rat cardiomyoblast cells (H9c2) were used for experimental studies.

Chemicals

Gallic acid was purchased from Merck, Türkiye. 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,9-dimethyl-1,10-phenanthroline (neocuproine), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ascorbic acid, quercetin, Folin-Ciocalteu's phenol reagent, potassium persulfate, sodium phosphate, ammonium molybdate, sodium nitro- prusside dihydrate, sulfanilamide, N-(1-naphthyl) ethylenediamine and other chemicals were purchased from Sigma, Türkiye.

Doxorubicin was obtained from Cayman, Türkiye.

Methods

Extracts Preparation

Air-dried and powdered inner perianth segments of the plant substances (5 g) were extracted with ethanol (3 x 50 ml) in a water bath at 40°C, concentrated to dryness under decreased pressure and lyophilized in vacuo. The extracts were dissolved in ethanol to prepare the required concentrations for antioxidant studies. For cytotoxicity studies, the extracts were dissolved in dimethyl sulfoxide (DMSO)

to prepare 10 mM stock solutions as an initial step, then the required concentrations were prepared via stock solution.

Resazurin Reduction Assay

We performed the resazurin reduction assay in order to examine the cytotoxicity of the extracts. The assay depends on the reduction of resazurin to resorufin by viable cells [30]. When non-viable cells lose their metabolic capacity hindering resorufin generation from resazurin, they do not show a blue staining. Briefly, aliquots of 5×10^5 adherent cells were seeded in 96-well-plates and were allowed to attach overnight. In the following, the cells were incubated with or without adding variable concentrations of the test substance to obtain a final volume of 200 µl/well. After 72 h incubation and combining resazurin (Sigma-Aldrich, Türkiye) for 4 h, staining was determined by an Infinite 200 M Plex plate reader (Tecan, Türkiye) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was independently performed thrice, with six parallel replicates each. The protocol has been recently reported [31].

Ascertaining Total Phenolic Contents

Phenolic contents of the inner perianth segments of plants were detected via Folin-Ciocalteau's reagent according to the protocol applied by Arıtuluk et al. (2006) with slight modification [32]. Folin-Ciocalteau's reagent was diluted with distilled water and then this solution was mixed either with the extract or with different concentrations of the reference compound. The mixture was mixed and sodium carbonate (Na_2CO_3) solution was joined. The reaction mixture was maintained at room temperature for 2 hours in darkness, then absorbance was determined at 765 nm. The assay was examined thrice. We used gallic acid as a reference. The total phenolic contents of the inner perianth segments were expressed as gallic acid equivalents (mg/g extract).

Antioxidant Assays

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The previously described method was applied to test DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity [19,33]. Ascorbic acid was used as a positive control.

ABTS (2,2′-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid) Radical Scavenging Activity

The previously described method with slight modifications was performed according to the previously described method to examine Trolox equivalent antioxidant capacity (TEAC) [19,34]. Trolox was used as a positive control.

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

The assay was applied according to the method described by Arıtuluk et al. (2006) with slight modification [32]. Gallic acid was used as a reference. Results were represented as gallic acid equivalents (mg/g extract).

Statistical Analysis

The resazurin reduction assay was carried out independently and performed thrice, with six parallel replicates each. Determination of phenolic content and antioxidant assays were conducted independently and performed thrice. Microsoft Excel was used for the generation of the graphs. The results were expressed as ± standard deviations (SD).

RESULT AND DISCUSSION

Cytotoxicity of the Ethanolic Extracts of *Rumex* Inner Perianth Segments Towards a Variety of Breast Cancer Cells

Our initial aim was to test the ethanolic extracts of *Rumex* inner perianth segments on assorted breast cancer cells with different origins. Therefore, as a preliminary evaluation, we tested all the

extracts at 30 µg/ml to see if they exhibited sufficient cytotoxicity deserving further detailed cytotoxic studies. The extracts were applied to various breast cancer cells including MDA-MB-231, MDA-MB-468, MCF-7 and SK-BR-3. The ethanolic extract of the *R. sanguineus* inner perianth segments exhibited the best cytotoxicity on SK-BR-3 cells among all the extracts with 57.10 ± 1.97% cell viability at 30 µg/ml, while others have relatively poor cytotoxic activity.

The cell viability percentages of the breast cancer cells under the treatment of the ethanolic extracts of *Rumex* inner perianth segments are shown in Table 2 and Figure 1 below.

Table 2. The effects of ethanolic extracts of *Rumex* fruits on breast cancer cells at 30 µg/ml

The ethanolic extracts of *Rumex* fruits	**The cell viability % on MDA-MB-231**	**The cell viability % on MDA-MB-468**	**The cell viability % on MCF-7**	**The cell viability % on SK-BR-3**
Rumex alpinus L.	86.35 ± 7.61	82.25 ± 5.82	84.66 ± 0.91	95.05 ± 13.78
Rumex amanus Rech.*	84.70 ± 9.63	86.73 ± 9.23	79.01 ± 11.18	86.31 ± 7.08
Rumex caucasicus Rech.	81.96 ± 8.16	74.55 ± 4.65	81.54 ± 9.57	81.48 ± 5.51
Rumex chalepensis Mill.	84.47 ± 6.37	80.96 ± 5.64	84.82 ± 10.40	69.76 ± 8.60
Rumex conglomeratus Murray	88.04 ± 12.18	77.47 ± 9.30	100.15 ± 7.85	76.78 ± 11.02
Rumex crispus L.	79.56 ± 7.78	70.91 ± 6.22	79.15 ± 5.42	65.01 ± 3.50
Rumex cristatus DC.	80.18 ± 12.08	73.82 ± 6.85	78.22 ± 10.88	69.03 ± 5.94
Rumex grasilescens Rech.*	85.36 ± 6.98	77.30 ± 2.54	73.12 ± 15.76	74.51 ± 8.41
Rumex hydrolapathum Huds.	80.80 ± 6.93	80.88 ± 7.43	86.96 ± 4.95	73.31 ± 8.23
Rumex sanguineus L.	80.20 ± 9.52	79.32 ± 5.52	74.87 ± 9.32	57.10 ± 1.97
Rumex tmoleus Boiss.*	80.43 ± 3.71	84.04 ± 8.02	88.78 ± 8.20	83.58 ± 9.14
Rumex obtusifolius subsp. *subalpinus* L.	84.35 ± 2.70	73.55 ± 7.05	77.97 ± 4.04	96.05 ± 12.13
Rumex olympicus Boiss.*	85.55 ± 8.63	79.36 ± 11.51	82.51 ± 3.17	83.56 ± 3.60
Rumex pulcher L.	83.99 ± 7.88	82.07 ± 5.01	90.68 ± 7.00	78.58 ± 3.32

* Endemic plant

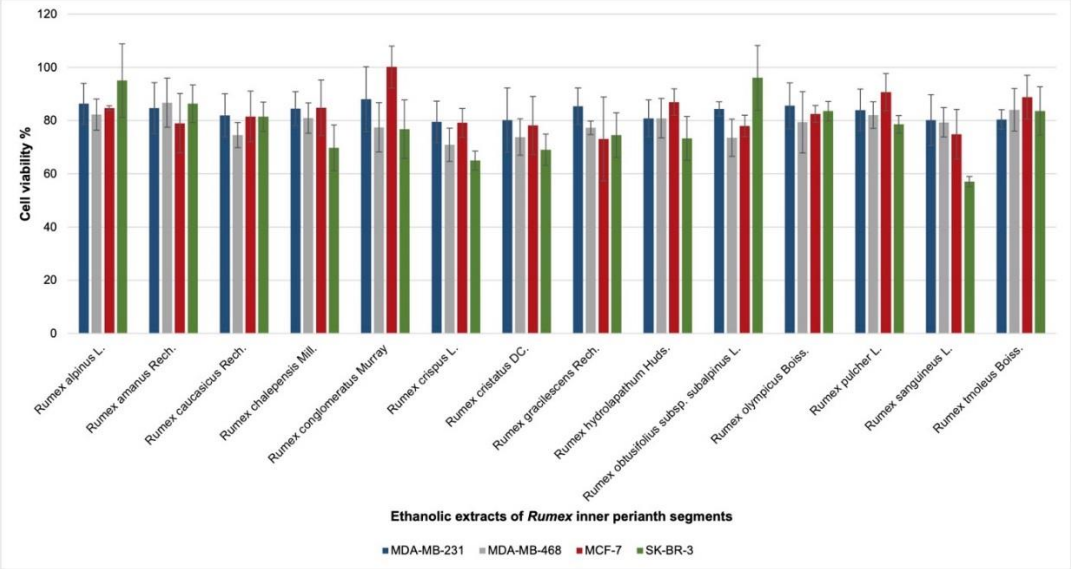

Figure 1. The effects of the ethanolic extracts of *Rumex* inner perianth segments on a variety of cancer cells at 30 µg/ml

Toxicity of the Ethanolic Extracts of *Rumex* Inner Perianth Segments in Normal Cells

We also investigated the toxicity of the ethanolic extracts of *Rumex* inner perianth segments on healthy rat cardiac H9c2 myoblastoma cells. Assessing their toxicity at 30 µg/ml, we observed that the extracts did not exert remarkable toxicity on H9c2 cells (Figure 2). Cardiotoxicity is one of the most common and serious side effects of clinically used chemotherapeutics such as doxorubicin [35-37]. Therefore, our assumption was cytotoxic agents with low toxicity on healthy cells may keep promise either as potential drug leads or as a part of drug combination regimens. Nearly all the tested extracts possessed low toxicity confirming their safety profile in comparison to clinically used doxorubicin which killed nearly half of the cells at the lowest concentrations tested (0.003 µM) (Figure 2).

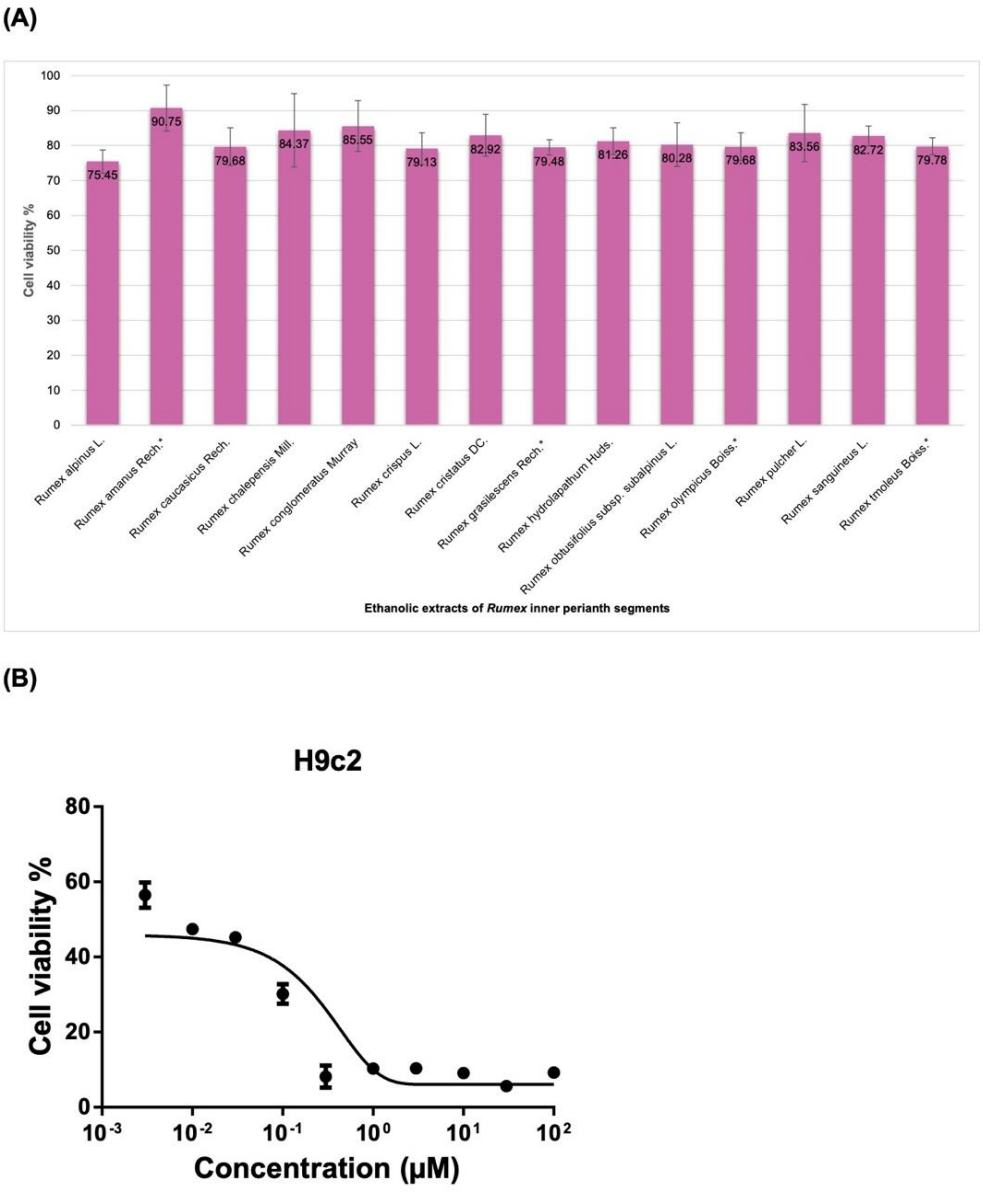

Figure 2. The effects of **(A)** the ethanolic extracts of *Rumex* inner perianth segments on H9c2 rat cardiac myoblastoma cells at 30 µg/ml **(B)** doxorubicin on H9c2 rat cardiac myoblastoma cells at various concentrations

Ascertaining Total Phenolic Contents

We investigated the total phenolic contents of the extracts. Total phenolic contents were estimated according to the equation (y=0.0042x+0.5131, R^2=0.9957) acquired from the calibration curve of gallic acid. The results were expressed as mg gallic acid equivalent (GAE)/g extract). The amount of total phenolics in the extracts ranged from 54.60 to 747.05 mg GAE/g extracts (Figure 3). The highest total phenolic grades have been observed in *R. conglomeratus*, while the lowest activity was detected in *R. alpinus*, confirming the outcomes of DPPH, ABTS and CUPRAC tests.

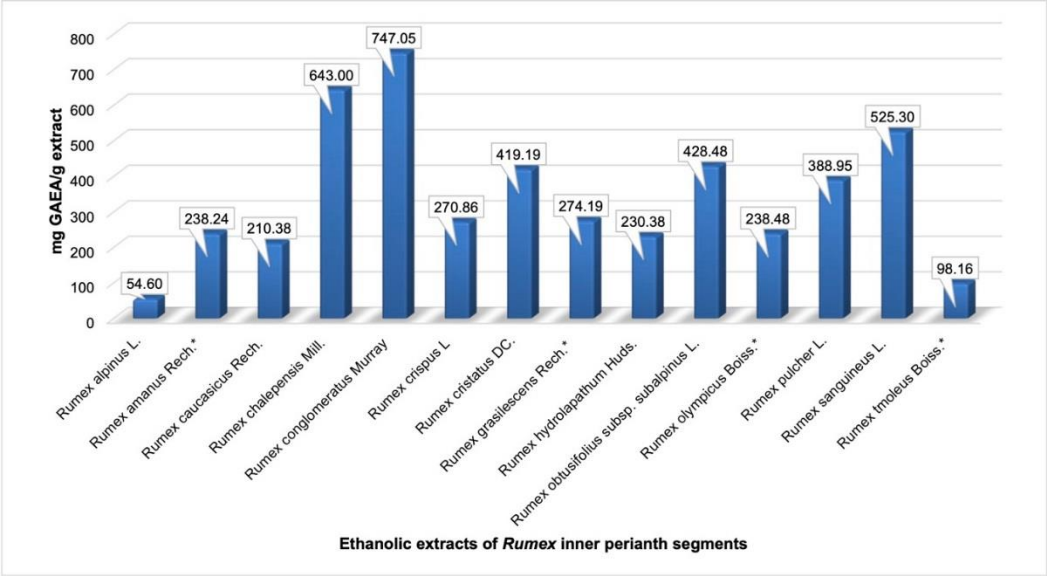

Figure 3. Total phenolic content of the ethanolic extracts of *Rumex* inner perianth segments (mg GAE/g extracts)

Antioxidant Assays

DPPH Radical Scavenging Activity

The assay is a method in which antioxidants donate hydrogen atoms resulting in the formation of reduced form of DPPH radical. DPPH generates purple/violet color in an alcohol solution and discolors the shades of yellow color in the presence of antioxidants [38]. The DPPH radical scavenging activities of the ethanolic extracts of *Rumex* inner perianth segments were tested at 6.25, 12.5, 25, 50 and 100 µg/ml concentrations. All the extracts scavenged DPPH radical dose-dependently. 50% radical scavenging capacities (RC_{50}), the concentration in which the extracts scavenge 50% of the DPPH radical, were stated in Figure 4.

The plant extract with higher antioxidant activity possessed a lower RC_{50} value in comparison to those with lower antioxidant activity. Almost all the extracts except for the ethanolic extract of *R. alpinus* inner perianth segments (RC_{50}: 75.07 µg/ml) efficiently scavenged DPPH radical with low RC_{50} values similar to that of reference compound ascorbic acid. Furthermore, the ethanolic extracts of *Rumex obtusifolius* subsp. *subalpinus* (RC_{50}: 7.83 µg/ml), *R.crispus* (RC_{50}: 10.91 µg/ml), *R. conglomeratus* (RC_{50}: 12.64 µg/ml) *R. pulcher* (RC_{50}: 13.02 µg/ml) and *R. olympicus* (RC_{50}: 14.06 µg/ml) inner perianth segments possessed higher DPPH radical scavenging activity than that of ascorbic acid with the RC_{50} of 14.44 µg/ml, emphasizing the importance of *Rumex* inner perianth segments as potential antioxidant agents.

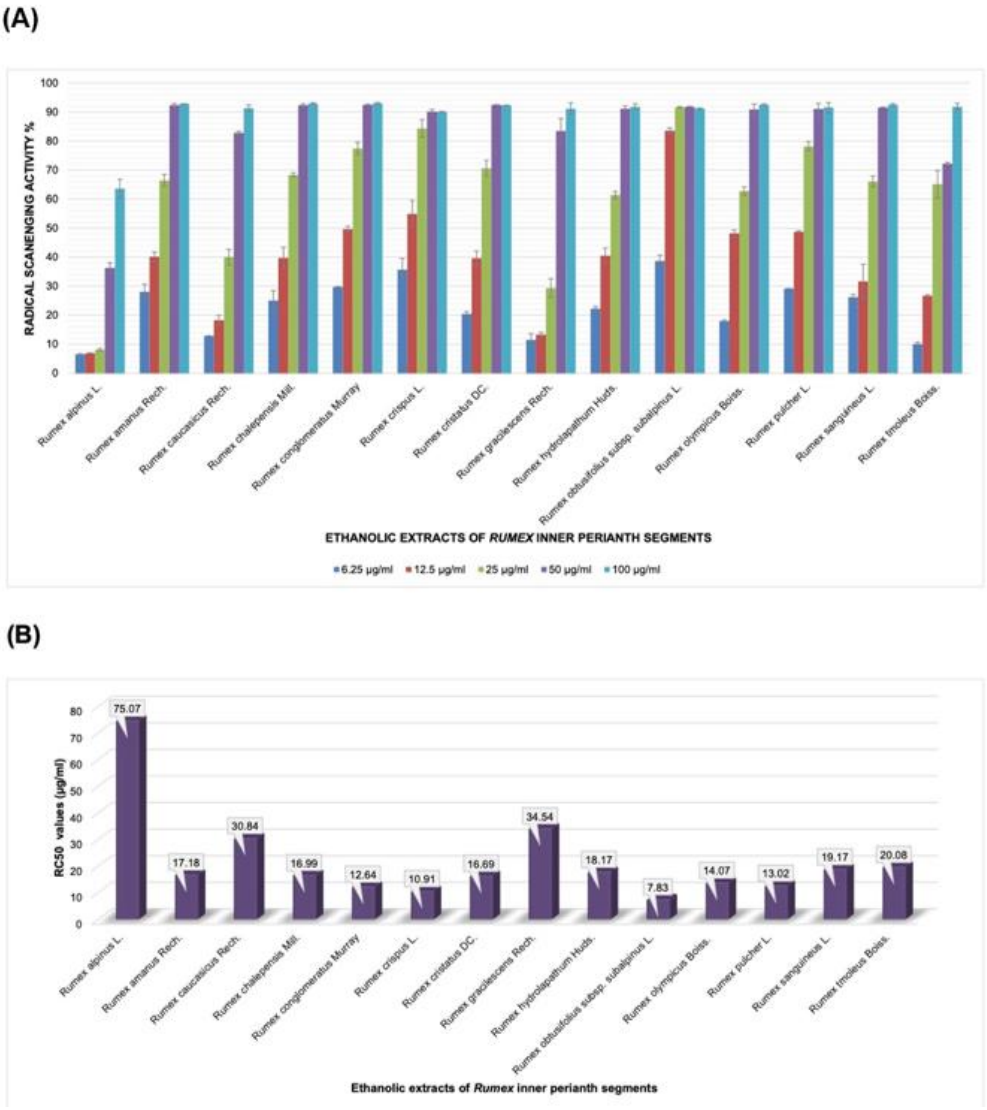

Figure 4. DPPH radical scavenging activity % of the ethanolic extracts of *Rumex* inner perianth segments **(A)**. RC_{50} values of the ethanolic extracts of *Rumex* inner perianth segments (**B**)

ABTS Radical Cation Scavenging Activity

ABTS radical cation scavenging activity assay is a method that depends on the neutralization of the ABTS radical cation in the existence of antioxidants and is widely used for the preliminary determination of antioxidant capacities of natural products [39,40]. ABTS radical cation scavenging activity of the ethanolic extracts of *Rumex* inner perianth segments was ascertained in reference to the equation ($y=1.8032x+1.7793$, $R^2=0.9845$) of the Trolox calibration curve. The results are demonstrated in terms of TEAC in Figure 5. A greater antioxidant activity of the extract was indicated with higher TEAC values. Therefore, evaluating TEAC of the extracts, specifically the ethanolic extracts of *R. conglomeratus* and *R. sanguineus* possessed the highest TEAC with the values of 2154.08 and 2036.94 mg Trolox/g extract, respectively. Similar to DPPH assay, *R. alpinus* possessed the lowest TEAC 393.63 mg TE/g extract). The rest of the extracts exhibited identical ABTS radical cation scavenging activities with similar TEAC values (Figure 5).

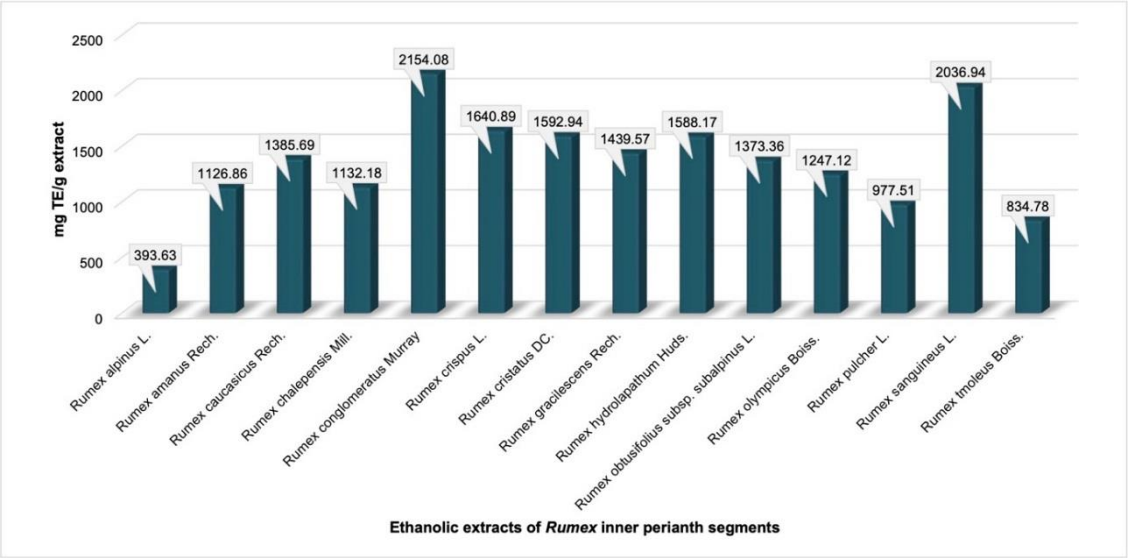

Figure 5. ABTS radical cation scavenging activity of the ethanolic extracts of *Rumex* inner perianth segments (mg TE/g extract)

CUPRAC Assay

Antioxidants do not only scavenge free radicals by donating electrons but also ease higher valent atoms to their lower valence state (e.g. iron, copper… etc.). The redox potential of an antioxidant gets an interest and is involved in the activity [41]. In the case of CUPRAC assay, the reducing power of antioxidants to convert cupric (Cu^{+2}) to cuprous (Cu^{+1}) ion is measured [42]. Cupric ion-reducing antioxidant capacities of the extracts were unraveled according to the equality (y=0.0099x+0.4928, R^2=0.9939) as gallic acid equivalent (mg/g extract). The results are shown in Figure 6. The CUPRAC values of the extracts ranged from 16.57 to 809.49 mg GAE/g extract. *R. obtusifolius* subsp. *subalpinus* displayed the highest antioxidant capacity, which was rather like DPPH radical scavenging activity outcomes. On the other hand, the ethanolic extracts of *R. alpinus* inner perianth segments did not exhibit any antioxidant activity, and the ethanolic extracts of *R. amanus* inner perianth segments displayed slight cupric ion-reducing antioxidant capacity (16.57 mg GAE/g extract).

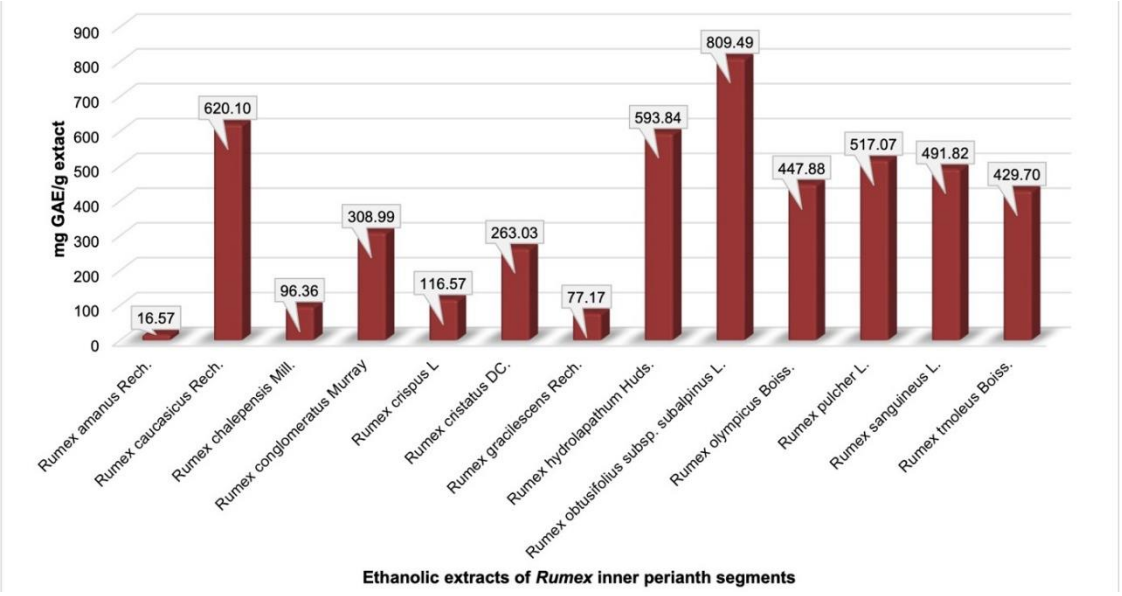

Figure 6. CUPRAC (Cupric ion reducing antioxidant capacity) of the ethanolic extracts of *Rumex* inner perianth segments (mg GA/g extract)

According to the National Cancer Institute USA (NCI), botanicals are taken into consideration as efficient cytotoxic agents if their IC_{50} values are below 20 μg/ml upon 72 h incubation [43,44]. Besides, NCI suggests that botanicals yielding IC_{50} values around or below 30 μg/ml should be subjected to purification to obtain cytotoxic molecules [44,45]. To be on the safe side, we set 30 μg/ml as a cut-off value for determining the extracts with effective cytotoxicity worth further investigations.

Unfortunately, nearly all the extracts studied within the context of our study showed no remarkable cytotoxic activity despite becoming relatively safe agents based on the preliminary evaluation of resazurin data.

Phenolic compounds (PCs) are extensively distributed phytochemicals in plants. They are secondary metabolites biosynthesized through the shikimic acid and phenylpropanoid pathways [46]. PCs exhibit various biological properties and, their dietary intake supplies beneficial effects on health [47]. Many studies emphasized their contribution to antioxidant activity [48]. Oxidative stress arises from the imbalance between the formation and the accumulation of reactive oxygen species (ROS) and is the underlying cause of many diseases [49]. Therefore, scavenging ROS by plants rich in phenolics may be a rational strategy to prevent or treat many disorders. Therefore, we also assessed the presence of phenolics and the antioxidant properties of the extracts.

The outcomes of this research demonstrated that generally, all the extracts have antioxidant activity. Among these *R. conglomeratus* possessing the highest total phenolic contents comes to the forefront also by exhibiting one of highest antioxidant activity by DPPH radical and ABTS radical cation scavenging activities. On the other hand, the ethanolic extract of *R. alpinus* inner perianth segments exhibited the lowest total phenolic and antioxidant properties in all.

Our study also holds importance in that 4 endemic species (*R. amanus*, *R. grasilescens*, *R. tmoleus*, *R. olympicus*) were investigated and assessed for the first time in terms of their antioxidant and cytotoxic profiles.

In this research, we evaluated the cytotoxic and antioxidant profiles of 14 *Rumex* species grown in Türkiye. We selected a variety of breast cancer cells with different origins to test the cytotoxicity of the ethanolic extracts of *Rumex* inner perianth segments. A number of investigations have been carried out to determine the cytotoxic potential of assorted *Rumex* species on various cancer cells. Emerging evidence has pointed out the cytotoxic activity of some *Rumex* species on assorted cancer cell lines to date. Ahmad et al. (2016) stated the potent cytotoxicity of the chloroform fraction of *R. hastatus* on HeLa and NIH/3T3 cells, emphasizing the importance of the fraction for further studies to isolate the potential cytotoxic compounds [49]. In another study, the dichloromethane extract of *R. crispus* effectively inhibited the cell viability of MCF-7 cell line [50]. Li et al. (2022) compiled the current literature data and mentioned the antitumor properties of various *Rumex* species such as *R. acetosa*, *R. thyrsiflorus, R. crispus, R. rothschildianus …etc.* on a variety of cancer cells [2].

The present study assessed the cytotoxic and antioxidant profiles of 14 *Rumex* species grown in Türkiye. We selected a variety of breast cancer cells with different origins to test the cytotoxicity of the ethanolic extracts of *Rumex* inner perianth segments. A number of investigations have been examined to determine the cytotoxic potential of assorted *Rumex* species on assorted cancer cells. Emerging evidence has pointed out the cytotoxic activity of some *Rumex* species on assorted cancer cell lines to date. Ahmad et al. (2016) stated the potent cytotoxicity of the chloroform fraction of *R. hastatus* on HeLa and NIH/3T3 cells, emphasizing the importance of the fraction for further studies to isolate the potential cytotoxic compounds [49]. In another study, the dichloromethane extract of *R. crispus* effectively inhibited the cell viability of MCF-7 cell line [50]. Li et al. (2022) compiled the current literature data and mentioned the antitumor properties of various *Rumex* species such as *R. acetosa*, *R. thyrsiflorus, R. crispus, R. rothschildianus …*etc. on a variety of cancer cells [2].

Despite the presence of various studies in the current literature data, no research is available comparing the cytotoxic activity and antioxidant profiles of *Rumex* inner perianth segments. Though there are few studies unraveling the cytotoxic activity of a particular *Rumex* plant of interest, they do not provide sufficient data to come to a general conclusion about the cytotoxicity of *Rumex* inner perianth segments on breast cancer cells. Our study holds importance in that 14 *Rumex* inner perianth segments were tested on four breast cancer cells with different origins. To be more precise, we selected

the breast cancer cells based on their origin (i.e., whether they carry ER, PR, or HER2/neu or not), and tested a series of *Rumex* inner perianth segments on those cells. Thus, unlike the existing data in the literature, which includes assorted extracts or subfractions of some *Rumex* species, we intended to give a general concept about the cytotoxic evaluation of the ethanolic extracts of 14 different types of *Rumex* inner perianth segments on breast cancer cells. Further, we also examined and compared 14 different types of *Rumex* inner perianth segments in terms of their antioxidant activity. Considering that antioxidant activity is involved in the pathogenesis of many diseases including cancer, our research demonstrated the potential of *Rumex* inner perianth segments as probable complementary and alternative agents, which can be used in cancer therapy as combination regimens.

The phytochemical compositions of the plant directly contribute to their activity. The description and identification of the plant extracts are needed for a detailed investigation of *Rumex* species of particular interest. In the HPLC studies performed on the inner perianth with fruit in our previous doctoral thesis, especially in *R. crispus* and *R. conglomeratus* species, hyperoside from flavonoids was found to be high. It was determined that the inner perianth of *R. tmoleus* and *R. tuberosus* is rich in frangulin B and carries a higher rate than all other species. Frangulin B was found as the main ingredient in the inner perianth segments of the subgenus *Rumex* [51]. Our group has been focusing and specializing on *Rumex* species, their phytochemical composition, and their biological activities for 30 years. Our research is still ongoing. We are also about to publish new findings on the cytotoxicity of *Rumex* roots and *Rumex*-containing constituents as well as their relevant modes of action. In the present study, we give an overview of the cytotoxic and antioxidant activities of the ethanolic inner perianth segment extracts of 14 *Rumex* species for the first time. We believe our findings will make a significant contribution to the assessment and comparison of the bioactivities of *Rumex* inner perianth segments.

To conclude, the ethanolic extracts of 14 *Rumex* inner perianth segments grown in Türkiye were examined in terms of their cytotoxicity on breast cancer, toxicity on H9c2 healthy rat myoblastoma cells, and phenolic contents and antioxidant properties for the first time. They did not exhibit remarkable cytotoxicity profiles on breast cancer cells despite being safe on healthy cells. On the other hand, they generally possessed rich phenolic content and high antioxidant capacity.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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