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# Evaluation of the antimicrobial activity and cytotoxicity of *Rhaponticoides iconiensis* seed extract

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## ABSTRACT

The goal of this study is to evaluate the antimicrobial and cytotoxic activities of aqueous extract from *Rhaponticoides iconiensis* seed. The Soxhlet extraction method was used to extract the seed in distilled water. It was tested for antimicrobial activities against pathogenic bacteria Escherichia coli using the disc diffusion method. Additionally, the cytotoxic activity of the seed extract on the MCF7 breast cancer cell line was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. *R. iconiensis* showed strong antibacterial activity against Escherichia coli compared to Cefotaxime antimicrobial agents. Additionally, an effective *in vitro* cytotoxic activity against the MCF7 is observed ($p<0.01$). The present study is the first report of endemic *Rhaponticoides iconiensis* seeds exhibiting potential antimicrobial activity and cytotoxicity, and it requires further investigation and characterization. These findings may be applied as a guideline for selecting Turkish medicinal plant species for further pharmacological and phytochemical studies.

**Keywords:** *Rhaponticoides iconiensis*, antimicrobial, cytotoxicity, cancer, phytotherapy

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## INTRODUCTION

Several plants are regarded as potential candidates for drugs due to their drug-like characteristics[1]. Since Türkiye is included in three different flora regions, plant diversity is of great importance. Many plants belonging to these regions are distributed among the plant communities in Türkiye[2,3]. It is known that there are approximately 9000 different natural plant species in Türkiye and 30% of these plants are endemic. Although there are many endemic plants, this diversity of plants cannot be utilized sufficiently[4].

Cancer is the primary cause of mortality in every nation worldwide[5]. Based on 2019 estimates, the World Health Organization (WHO) indicated that in 112 of 183 countries, cancer was the first or second most common cause of death before the age of 70, and in 23 countries, it was the third or fourth leading cause[6]. Although there is a significant decrease in stroke and coronary heart disease death rates compared to cancer, this is still the leading cause of death[7]. GLOBOCAN 2020 estimated that cancer claimed 10 million lives with 19.3 million new cases diagnosed in 2020[8].

Asteraceae is a vast and globally distributed family of flowering plants, with over 1,100 genera and 2,500 species. Within this family, *Centaurea L.* genus is among the largest and the most significant genera within the Asteraceae family and the *Rhaponticoides* genus is a member of the Asteraceae family. *Rhaponticoides iconiensis* (*R. iconiensis*), a species within this genus, is endemic to Konya, Türkiye[9]. It is widely used as herbal medicine due to its various properties[6]. In Türkiye, there are 8 species belonging to the *Rhaponticoides* genus with 7 of them being endemic. These species are known by the Turkish name 'Tülüşah'[4,10]. In our study, we focused on *R. iconiensis* which is one of these species.

There are few studies that reported chemical composition and bioactivity of *R. iconiensis*. Paşayeva et al. showed the antioxidant and antidiabetic activity of *R. iconiensis* flower[11]. Additionally, antioxidant properties, total phenol amounts and flavonoid amounts of the endemic *Rhaponticoides* species were determined. The mean percentage of DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant free radical scavenging effect values is presented[12]. The antioxidant properties, enzyme inhibition, and levels of phenolics and flavonoids in methanol extracts (obtained via maceration and Soxhlet extraction) and extracts of water (prepared through infusion) were assessed of leaves, roots, and flower heads of *R. iconiensis*. It was reported that the leaf extracts of *R. iconiensis* had higher levels of phenolic and flavonoid compounds compared to

the flower and root extracts. Also, the extract had 87 compounds, including hydroxybenzoic, hydroxycinnamic, and acylquinic acids, anthocyanins, flavones and flavanones. On the other hand, it has been indicated that *R. iconiensis* might be a valuable source of natural enzyme inhibitors for developing new drugs to address global health issues because of its enzyme inhibitory effects[13].

Despite all aforementioned data, there is no study evaluated effect of *R. iconiensis* seed extract. We aimed to uncover the antimicrobial activity and cytotoxicity of aqueous *R. iconiensis* seed extract.

## METHODOLOGY

### Plant material

The endemic *R. iconiensis* (Hub.-Mor.) M.V. Agab. & Greuter (Voucher No. 11.048) used in our studies was obtained from the Nezahat Gökyiğit Botanical Garden with its species identification confirmed. A very small population of *R. iconiensis* was found between the canal and the highway near the village of Orta Karaören, 18 km from Seydisehir, by the expert of the subject Prof. Dr. Mecit Vural.

### Solvent extraction of plants

*R. iconiensis* seeds were weighed to 10 g in a Soxhlet extractor thimble and placed in the extraction apparatus. Seeds were extracted in a 250 ml conical flask based on the feed-to-solvent ratio [1:10 (w/v)]. The extraction was performed on 10 g of seed using 100 ml of water. A heating mantle was utilized to reflux the mixture for extraction periods spanning from 6 to 8 hours[13]. Once the extraction time was completed, the extract solution was cooled at room temperature. It was subsequently filtered using cone filter paper (Whatman no.1) and left in a water bath[14]. Powder sample was measured and stored at -4°C for further analysis.

### Cell culture and cell line

For the assessment of the anticancer activity of the seeds, MCF7 human breast cancer cell line was used. L929 mouse fibroblast were used as a negative control in the study. MCF7 and L929 cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture (DMEM) medium supplemented with 10% FBS (Fetal Bovine Serum), 1% penicillin-streptomycin (P4458; Sigma, USA) and 1% L-glutamine at 37°C with 5% $CO_2$ in a humidified incubator. Cells were suspended in medium with 1% penicillin-streptomycin (P4458; Sigma, USA) and 1% L-glutamine. Then, cells were counted (at a density of $2\times10^4$ cells/ml), transferred into a 96-well plate, and incubated for 24 hours prior to the addi-

tion of extract. The seed extracts were dissolved in cell culture medium, then diluted to different serial concentrations. The cells were treated with 10, 50, 100 μg/ml dilutions of the seed extracts and 1% Triton-x was added as a positive control. Untreated cells given the same volume of medium considered as the control.

**Cell viability assay**

For cell viability assay, it was measured using the standard colorimetric MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay technique. After 24 hours incubation, 10 μL of MTT solution (5 mg/ml) was dispensed into each well and incubated for 4 hours. Subsequently, 100 μL of DMSO was added to each well to dissolve the formazan crystals. Then absorbance readings were recorded at 570 nm using a microplate reader for each cell line. The cytotoxicity was determined by comparing the absorbance levels between the serial dilutions and the control samples[15].

**Antimicrobial assay**

*Escherichia coli* (*E. coli*) (ATCC 25922) was obtained from the Department of Clinical Microbiology, Faculty of Medicine at Istanbul University. The organisms were kept in Nutrient agar at 4°C until analyses.

**Disc diffusion method**

The antimicrobial effect of *R. iconiensis* was tested according to the disc diffusion Kirby-Bauer technique, which complies with the standards recommended by CLSI, 2015[16]. To prepare the bacterial inoculum, the bacterial strain was suspended in sterile $dH_2O$, and the turbidity was adjusted to approximately $10^6$ CFU/ml, equivalent to 0.5 McFarland standards[17]. Nutrient agar (4018102; Biolife, Italy) was used for antimicrobial activity. Next, 28 g of agar was dissolved in 1 liter of $dH_2O$, autoclaved and allowed to cool. Then, 20 ml of agar was poured into petri dishes. The sterile swab was immersed in the standardized bacterial suspension and utilized to uniformly inoculate the bacteria onto Nutrient agar plates. The plates were left to air dry for 5 minutes and Whatman no.1 filter paper was utilized to produce discs with a diameter of 6 mm. These discs were sterilized via autoclaving and subsequently dried in a hot air oven at 80°C for one hour. Circular discs measuring 6 mm in diameter were created by punching through a sheet of filter paper. Each disc was impregnated with 20 μl each of 1 g/ml *R. iconiensis* aqueous extract, Cefotaxime antimicrobial agents (dissolved in $dH_2O$) as a positive control, or $dH_2O$ alone as a negative control. Following impregnation, all papers were dried, and the discs were maintained under sterile conditions until further use. Then, all discs were positioned on

the plates with flamed forceps and delicately pressed to guarantee complete contact with the agar. The plates were subsequently placed in an incubator at 37°C for a period of 24 hours. After incubation, the areas surrounding the discs where bacterial growth was impeded were measured and recorded in millimeters. The experiments were replicated six times to ensure consistency and reliability.

### Statistical analysis

Statistical evaluations of the results were performed using the ANOVA test with GraphPad InStat (GraphPad Software Inc., San Diego, CA, USA). Tests were conducted with a 95% confidence interval, and $p<0.05$ was considered significant. Data averages for the groups are presented as means ± standard deviation (SD). To determine the concentration necessary for a 50% reduction in cell viability ($IC_{50}$), regression analysis was used, and graphs were prepared using Microsoft Office Excel 2017.

## RESULTS and DISCUSSION

Many studies show that plants have a potential for use in traditional medicines in numerous countries worldwide. Moreover, plants are identified as a crucial source for discovering new cytotoxic compounds, with many polyphenolic flavonoids having antitumor effects[18]. Every part of the plant, including the leaf, flower, seed, and rhizome, is known to be used for both nutritional and medicinal purposes[19]. Extracts from plant flowers, fruits, and seeds have shown a variety of biological activities in numerous studies[20,21]. Despite the limited research has been conducted on endemic *R. iconiensis* to investigate its biological activities with some parts of it, there is an absence of studies regarding the activity of the seed. Herein, we document the cytotoxic and antimicrobial effects of seed samples from the endemic *R. iconiensis* species to elucidate their biological activities.

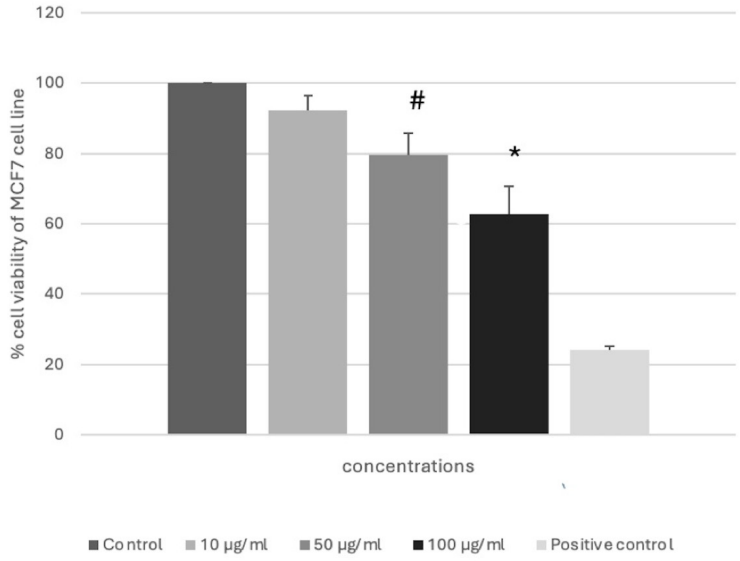
### Cytotoxic activity findings

In our study, MTT test was performed to reveal the cytotoxic effects of the aqueous seed of endemic *R. iconiensis* species on the MCF7 breast cancer cell line.

Breast cancer is the leading cause of death and the most prevalent type of cancer among women globally, with over 10 million new cases identified annually. The disease's progression, prognosis, and treatment outcomes are influenced by its heterogeneity. According to the WHO, breast cancer has the highest prevalence worldwide. In 2018, breast cancer caused to 2.09 million diagnoses

and 627,000 deaths among women. Moreover, around 1.7 million new cases were reported in 2020, resulting in an estimated 627,000 deaths[22]. Therefore, we showed that *R. iconiensis* could be a new potential candidate for MCF7 breast cancer cell line.

In a study, it is investigated the methanol extracts and sub-extracts derived from the leafy stems and flowers of *R. iconiensis*. Their cytotoxic effects were examined on A549, Colo 205, HepG2, Beas-2b and MCF7 cell lines using the MTT assay. The constituents of *R. iconiensis* extracts were identified and quantified using LC–MS/MS. Consequently, while the methanol extracts had no cytotoxicity against A549 cells, they showed cytotoxic effects on HepG2, Colo 205, MCF-7, and Beas-2b cell lines[23]. To determine whether seed of it has anti-cancer effect, we first explored the cytotoxicity of aqueous *R. iconiensis in vitro* and we showed the decreased % viability rate of the MCF7 cell line, and the results were statistically significant with increasing dose (Figure 1).




*p<0.01 compared to the control group

**Figure 1.** Representing the percentage (%) cell viability of *R. iconiensis* on MCF7 cell line. p<0.05. Values in the groups are expressed as mean (X) ± standard deviation (SD).

To investigate the cytotoxic effects of *R. iconiensis,* it was tested on healthy cell lines. After treating the L929 mouse fibroblast cells with different concentration of seed extract, there was no significant difference in viability of L929 compared to control (Figure 2).

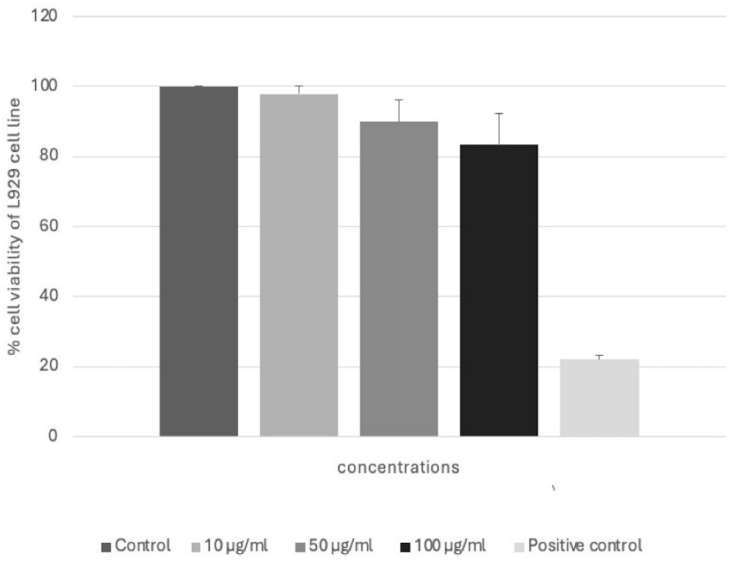



**Figure 2.** Representing the percentage (%) cell viability of *R. iconiensis* on L929 cell line. Results compared to control group. Values in the groups are expressed as mean (X) ± standard deviation (SD).

The $IC_{50}$ (the dose that inhibits cell growth by 50%) value of *R. iconiensis* was found 56.33 µg/ml in MCF7 cell line. Tugay et al. provided that sub-extracts of leafy stem and flowers of *R. iconiensis* were most effective against MCF7 cancer cell line[23]. In a study, the cytotoxic effects of methanol extracts from the stem and flowers of *R. iconiensis* were showed against the A-549, HEPG2, MCF7, COLO-205, and BEAS-2b cell lines by MTT and SRB methods[24]. Hence, in present study we have proven that seed extracts of *R. iconiensis* have cytotoxic effect on MCF7 cell line.

Consequently, *R. iconiensis* showed cytotoxic effect on MCF7 cell line depending on increasing doses [50 µg/ml ($p<0.05$) and 100 µg/ml ($p<0.01$)] while it has not showed reduction in cell viability on L929 mouse fibroblast cell line.

### Antimicrobial activity findings

The type of *E. coli* used in molecular biology labs is a model commensal bacterium that is prevalent in the mammalian intestine. However, some strains of this species can cause serious illnesses in humans[25]. Given this, the purpose of the present study was to determine the antimicrobial activity of *R. iconiensis* against *E. coli* and its antimicrobial activity has been demonstrated compared to Cefotaxime antimicrobial agents (Table 1). Paşayeva et al. reported the similar positive results, and they showed the antimicrobial effect with the microdilution method using leafy stem extracts of *R. iconiensis*[26].

**Table 1.** Zones of inhibitions as shown by aqueous *R. iconiensis* seed extract against *E. coli.* Data represents Mean ± Standard error of mean (n=6).

| Disc diffusion zone diameters (cm) ± SD | | | |
|---|---|---|---|
| **Microorganism** | **Positive control (Cefotaxime)** | ***R. iconiensis*** | **Negative control ($dH_2O$)** |
| ***E. coli*** | 4.9 ± 0,25 | 3,2 ± 0,2 | — |

*( — = no zone)

In summary, we have described robust antimicrobial activity and cytotoxic effect of seed samples from the endemic *R. iconiensis* which is extracted in distilled water by Soxhlet extraction method, we used only aqueous extract and focused exclusively on a single cell line and one bacterial strain. Hence, additional research encompassing a wider variety of cell lines and bacterial strains will provide a more thorough comprehension of their therapeutic potential.

### STATEMENT OF ETHICS

No need for ethical approval for this study.

### CONFLICT OF INTEREST STATEMENT

The authors state that they have no conflicts of interest.

### AUTHOR CONTRIBUTIONS

All authors contributed to the work equally.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