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Investigation the Cytotoxic and Antimicrobial Effect of *Ranunculus poluninii*

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Abstract

Ranunculus plant species, one of the endemic plants, are utilized to treat a number of illnesses such as febrile diseases, rheumatism and inflammatory rashes due to their pharmacological and toxicological activities. Considering these properties, it was aimed to investigate the cytotoxic effects of *Ranunculus poluninii* on A549 and HCT116 cancer cell lines, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria and *Candida albicans* and *Candida glabrata* yeasts. For this purpose, the extract of the plant was obtained by methanol extraction. Stocks were prepared from this plant at different concentrations of 200 µg/mL, 100 µg/mL, and 50 µg/mL for A549 cancer cell line and 400 µg/mL, 200 µg/mL, 100 µg/mL, and 50 µg/mL for HCT116 cancer cell line and applied to the cell lines. The 3- [4,5-dimethylthiazol-2 il]-2,5-diphenyl-tetrazolium bromide (MTT) assay was performed to evaluate the viability of the cells. In addition, plant extracts prepared at different concentrations of 800 µg/mL, 400 µg/mL, 200 µg/mL, 100 µg/mL, and 50 µg/mL were applied to *Escherichia coli*, *Pseudomonas aeruginosa and Staphylococcus aureus*, *Candida albicans* and *Candida glabrata* and minimum inhibitory concentration (MIC) values were determined. Our results showed that *Ranunculus poluninii* was reduced cell viability to 83.7% at 100 µg/mL and 79% at 200 µg/mL on A549 and HCT116 respectively. In addition, it decreased both *Candida albicans* and *Candida glabrata* growth to 91.5% at 800 µg/mL and 95.1% at 50 µg/mL respectively. Our results suggest that *Ranunculus poluninii* has anticancer effect and antifungal activity.

1. Introduction

One of the most prevalent and complicated illnesses that endanger people's health worldwide is cancer [1] and the burden of cancer mortality and incidence is increasing rapidly [2]. Various studies and research have been conducted to treat cancer and one of these studies is plant-based anticancer studies. Plants are essential to human life and are necessary for survival. Many countries utilize plants and extracts of plants for therapeutic purposes in order to prevent and treat diseases that affect humans. The importance of herbal-derived products continues to increase due to their low cost, less side effects and easy availability [3].

The *Ranunculaceae* family, popularly known as buttercup and consisting of plants such as small shrubs and woody vines, has nearly 60 genera and 2500 species in the world and 19 genera and 203 species in Turkey [4]. Plants of the *Ranunculaceae*

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family are distributed worldwide, mostly in temperate regions of the northern hemisphere. About 720 species of this plant family are distributed throughout China and have been used in traditional Chinese medicine for many years. Plants in this family contain a wide variety of chemical constituents such as benzylisoquinoline alkaloids, ranunculin, triterpenoid saponin and diterpene alkaloids [5]. The plant species *Ranunculus* has been used in traditional medicine for the treatment of various diseases such as febrile diseases, rheumatism and inflammatory rashes due to its pharmacological and toxicological activities. The family *Ranunculaceae*, which consists of herbaceous perennial or annual plants, also includes plant species with specific toxicological and pharmacological activities [6]. Studies using plant extracts of *Ranunculus* species have shown that they have antibacterial, antiviral and antiprotozoal effects, as well as antioxidant and anticarcinogenic properties [7]. In a study, the antimicrobial activity of a toxin called protoanemonin, which is found in all of the *Ranunculaceae* family, was investigated and showed that *Ranunculus bulbosus* has a significant inhibitory effect on fungi and yeast species [8]. *Ranunculus* plant species have also been used in anticancer studies. In a study with *Ranunculus repens* L., dichloromethane fraction of the plant was applied to gastric cancer AGS and chloroform fraction to colon and rectal tumors HCT116. As a result, the plant fractions inhibited the growth of cancer cells in a dose-dependent manner and were found to be active in reducing tumor cell migration [9]. In another study, ovarian cancer cell lines A2780 and SK-OV-3 were treated with *Ranunculus ternati* strain and the plant alone showed a remarkable cytotoxic effect on cell proliferation [10].

Based on previous studies, *Ranunculus poluninii* is expected to show antimicrobial, anticancer and antifungal properties like plant species in the *Ranunculaceae* family. This study focused on investigating the anticancer, antibacterial and antifungal properties of the plant by applying the plant extract to A549 and HCT116 cancer cell lines, as well as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria, and *Candida albicans* and *Candida glabrata* yeasts.

2. Material and Method

2.1. Methanol extraction

Root, stem, and leaf parts taken from the *Ranunculus poluninii* were pulverized and macerated in 80% methanol for 72 hours in a shaking incubator. Following a 72-hour period, filter paper was used to filter the solution and transferred to petri dishes left to dry for one day. After being weighed, the dry extract was dispersed in dimethyl sulfoxide (DMSO). Before usage, the mixture was kept at 4°C in a refrigerator [11].

2.2. Cell culture and incubation

Stocks of *Ranunculus poluninii* extract at different concentrations of 200 µg/mL, 100 µg/mL and 50 µg/mL for A549 cancer cell line and 400 µg/mL, 200 µg/mL, 100 µg/mL and 50 µg/mL for HCT116 cancer cell line were prepared and applied to cell lines. At 37°C and 5% CO_2, all cells were cultured in Dulbecco's Modified Eagle Media (DMEM). $1x10^4$ cells were seeded to 96 well plate. Lastly, to enable cell adhesion, dishes comprising cells were subjected to incubation for 24 hours at 37 °C in a 5% CO_2 environment.

2.3. MTT Assay

The 3- [4,5-dimethylthiazol-2 il]-2,5-diphenyl-tetrazolium bromide (MTT) assay was used to detect cellular viability of cells. Cells in the 96 well plate left to grow then transferred out of the incubator, and 1X PBS was used to clean each well. Each well was treated with *Ranunculus poluninii* solutions prepared at different concentrations and incubated for 24 hours. The wells were added with an amount of MTT reagent equal to 10% of the total volume of the wells and incubated again for 2-4 hours. After incubation DMSO was added and plates were incubated in incubator for 15 minutes. Absorbances were measured in a spectrophotometer [12].

2.4. Antimicrobial activity

Candida albicans (ATCC MYA-2876) and *Candida glabrata* (ATCC 2001) were used for antifungal tests, and *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) were used for antimicrobial tests. BMD (Broth Microdilution) test was used for antifungal and antimicrobial MIC analyses. For the purposes of the antifungal and antimicrobial tests, *Ranunculus poluninii* stock solution was made using only DMSO. Next, multiple dilutions were prepared in 96-well plates by YPD (Yeast Peptone Dextrose) medium (2% glucose, 2% peptone, 1% yeast extract, pH 6.5) [13] for yeasts and LB (Luria-Bertani) broth medium (1% tryptone, 1% NaCl, 0.5% yeast extract, pH 7.0) [14] for bacteria. To achieve the necessary cell density and concentrations of the sample to be tested, cell solutions of bacteria ($1x10^6$ CFU/mL) and yeast ($1\text{-}5x10^5$ CFU/mL) were adjusted by sterile water. After incubation, the MIC was measured spectrophotometrically for yeasts at 530 nm and

visually assessed for bacteria. For yeasts, plates were incubated for 24 hours at 37 °C, and for bacteria, for 16–18 hours at the same temperature. The lowest sample concentration that resulted in at least a 50% decrease in growth relative to the control (no sample) cell group was used to calculate the minimum inhibitory concentration (MIC) for yeasts, whereas the lowest sample concentration that resulted in no discernible growth was used to calculate the MIC for bacteria.

2.5. Statistical analysis

SPSS statistical programming was employed to conduct the statistical analysis. The data is shown as mean ± SD. The numerical data's mean and standard deviations were provided as a consequence of the analyses.

3. Results and Discussion

In numerous countries, cancer is one of the primary causes of death [15]. Researchers have carried out numerous experiments to prevent and treat cancer. Plants have an important place in studies on cancer and has been used in alternative medicine for many years [16]. Compounds from plants have been an important source of the anti-cancer agents [17]. The best known example of anticancer drug is Paclitaxel which is obtained from the bark of *Taxus brevifolia Nutt* [18]. Curcumin is also a another plant with anticancer activity [19].

Because of its pharmacological and toxicological properties, the *Ranunculus poluninii*, which is used in this study, is used to treat a variety of illnesses in, including rheumatism, inflammatory rashes, and febrile diseases. Although studies on anti-cancer activities of *Ranunculus* plant species are limited, studies using plant extracts of *Ranunculus* species have revealed that these extracts have antioxidant and anticarcinogenic properties [6,7]. Based to a study, an extract from the *Ranunculus ternatus*, which is a member of the *Ranunculaceae* family, dramatically and dose-dependently reduced the viability of Jurkat cells and induced death of the MCF-7 cells [20]. In study with *Ranunculus repens* L., Thymoquinone (TQ), a bioactive component of the plant with anti-cancer properties, was utilized. In the study, this bioactive substance obtained from the plant induced cell apoptosis at a higher rate in human gastric adenocarcinoma cell lines compared to the control and showed a decrease in cell proliferation [21]. In another study with *Ranunculus repens* L., the anticancer properties of *Ranunculus repens* L. were investigated and the dichloromethane fraction of the plant was applied to gastric cancer AGS and the chloroform fraction to colon and rectal tumor HCT116 cells. As a result, it was shown that the plant fractions were active in inhibiting cell growth and reducing tumor cell migration [9]. Another study used the *Ranunculus ternati* strain on the ovarian cancer cell lines A2780 and SK-OV-3. It revealed a notable cytotoxic effect on the proliferation of the cells [10].

In this study, anti-cancer effect of *Ranunculus poluninii* extract on cell viability against A549 and HCT116 cell line conducted at several concentrations between 50 and 200 µg/mL for A549 and 50 to 400 µg/mL for HCT116. Cells were treated with *Ranunculus poluninii* extract for 24 hours. There are significant decrease in cell viability was observed when the A549 and HCT116 cell lines were exposed to *Ranunculus poluninii*.

In the HCT116 cell, the decline in cell viability was slightly greater than A549 cell line. Cell viability for A549 cell line was 87.5% at 200 µg/mL, 83.7% at 100 µg/mL, and 91% at 50 µg/mL, while HCT116 cell line, cell viability was 87.2% at 400 µg/mL, 79% at 200 µg/mL, 80.5% at 100 µg/mL, and 80.1% at 50 µg/mL (Figure 1).

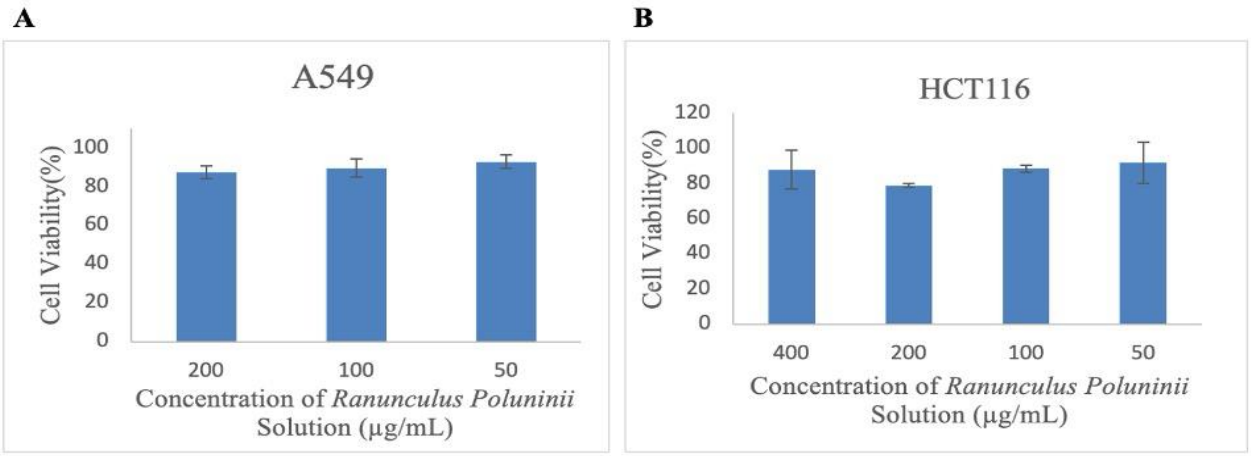

Figure 1: Effect of *Ranunculus poluninii*'s methanol extract on A549 (A) and HCT116 (B) cell lines.

In addition to the anticancer effect, *Ranunculaceae* family have antibacterial, antiviral and antiprotozoal effects [7]. Pathogenic yeast species *Candida albicans* and *Candida glabrata* were used for antifungal tests, and *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial species were used for antimicrobial tests. *Ranunculus poluninii* was applied at different concentrations range from 50 to 800 µg/mL against bacterial and yeast species. MIC analyzes were performed with reference to EUCAST E.DEF 7.3.2 for yeasts [22] and were carried out utilizing the BMD (Broth Microdilution) test as described in CLSI M07 [23] for bacteria.

As a result of antibacterial experiments, *Ranunculus poluninii* extract did not show antibacterial effects on *Escherichia coli* (Figure 2A), *Pseudomonas aeruginosa* (Figure 2B) and *Staphylococcus aureus* (Figure 2C). All bacteria continued to grow despite application of *Ranunculus poluninii* extract.

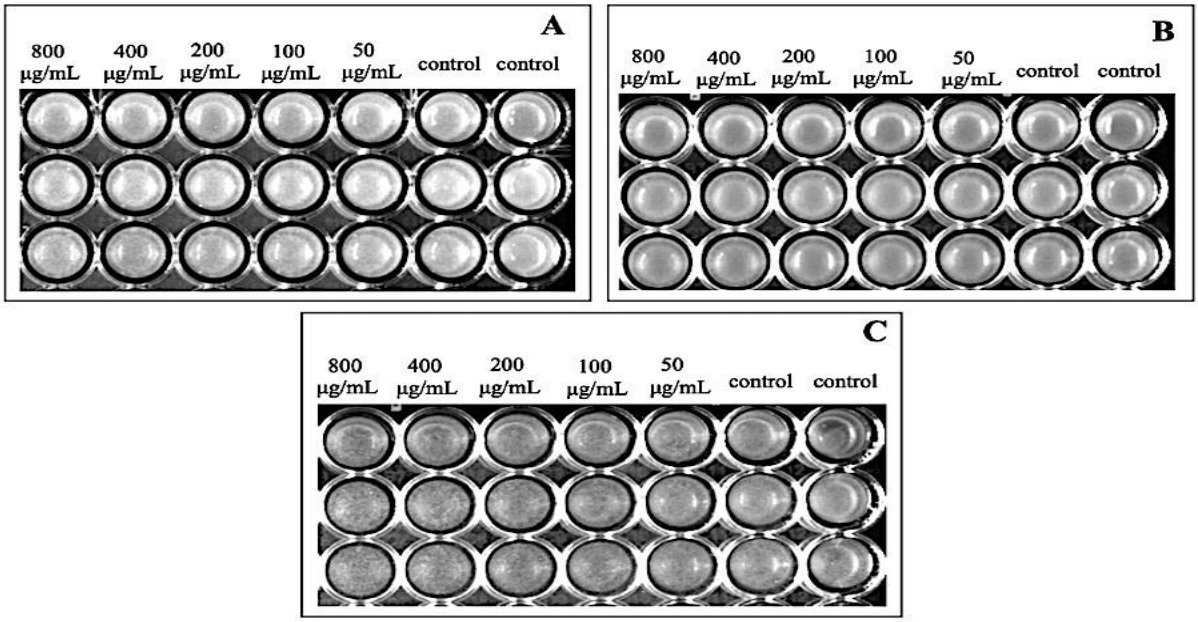

Figure 2. Antimicrobial activity of *Ranunculus poluninii's* methanol extract on *Escherichia coli* (A), *Pseudomonas aeruginosa* (B) and *Staphylococcus aureus* (C).

At the antifungal anaysis, cell viability for the *Candida albicans* was 91.5% at 800 µg/mL, 92.7% at 400 µg/mL, 95.2% at 200 µg/mL, 95.3% at 100 µg/mL, and 95.1% at 50 µg/mL, while the cell viability for *Candida glabrata* was 96.9% at 800 µg/mL, 97.2% at 400 µg/mL, and 96,3% at 200 µg/mL, 95.9% at 100 µg/mL, and 96.1% at 50 µg/mL (Figure 3).

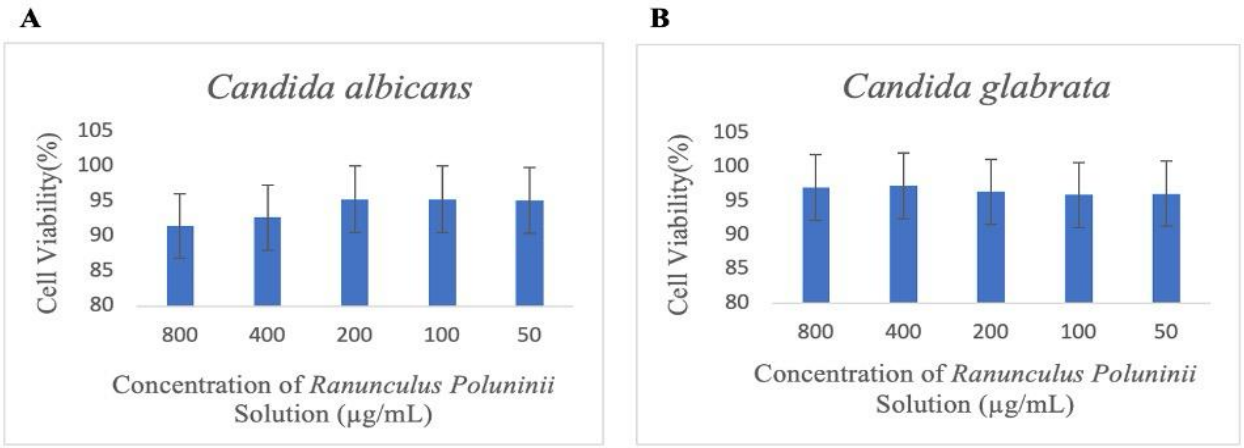

Figure 3. Effect of *Ranunculus poluninii's* methanol extract on *Candida albicans* (A) *and Candida glabrata* (B).

Previous studies have also demonstrated that a toxin called protoanemonin, which is found *Ranunculus bulbosus*, has been shown to have a significant inhibitory effect on fungi and yeast species. Among the tested strains, *Rhodotorula glutinis* was the most susceptible yeast and *Epidermophyton floccosum* was the most susceptible dermatophyte [8]. In a study investigating antimicrobial activity, nine test microorganisms including *Escherichia coli, Proteus sp., Pseudomonas aeruginosa, Shigelladysenteria, Salmonella enteritidis, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis* and *Candida albicans* were investigated by single disk diffusion method. The methanol extract of the whole plant of *Ranunculus myosuroudes* was reported to be active against 88.8% of the tested microorganisms [24]. In another study, antibacterial and antifungal activities of four fractions (n-hexane, chloroform, ethyl acetate and ethanol) of *Ranunculus muricatus* were tested against *Staphylococcus aureus, Micrococcus luteus* and *Escherichia coli, Pseudomonas aeruginosa, Enterobactercloacae, Klebsiella Pneumoniae* strains. Almost all fractions exhibited antimicrobial activity. Among them, the ethyl acetate fraction showed maximum antimicrobial activity against *Staphylococcus aureus* [25].

Nevertheless, no inhibitory effect was observed by *Ranunculus poluninii* extracts on *Escherichia coli*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus* in this investigation. However, unlike bacteria, the results of previous studies on yeast in the literature are similar to the present findings in this study. The application of *Ranunculus poluninii* methanol extract was found to reduce the cell viability of *Candida glabrata* and *Candida albicans*.

4. Conclusion and Suggestions

In conclusion, our findings indicate that *Ranunculus poluninii* exhibits anticancer properties on A549 and HCT116 cell lines, as well as antiyeast activity against *Candida albicans* and *Candida glabrata*. Nevertheless, additional investigation is required to precisely understand the impact of *Ranunculus poluninii* on cancer cell lines and yeast species.

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Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

Conflict of Interest Statement

There is no conflict of interest between the authors.

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