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Research Article

Investigation the Biological Activities of *Chenopodium foliosum* Methanol Extract

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Abstract: The genus *Chenopodium* is distributed almost all over the world, including Turkey. The species belonging to this genus are used in the traditional medicine of different countries in the treatment of various diseases. Different species of the genus *Chenopodium* show anticancer, antifungal, and antibacterial effects. There is a significant gap in the literature regarding *Chenopodium foliosum (C. foliosum)*. In this study, the biological activities of the methanol extract of *C. foliosum* were investigated. The anticancer activity was evaluated using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay against HCT116 and A549 cell lines. The antibacterial and antifungal activity were examined using the minimum inhibitory concentration (MIC) assay. Methanol extract of *C. foliosum* reduced cell viability of HCT116 and A549 cell lines to 73.4 ± 3.3 % and 78.7 ± 2.3 % respectively. *C. foliosum* also decreased the viability of the *Candida albicans (C. albicans)* to 91.2 %. Our research suggests that the methanol extract of *C. foliosum* slightly reduces the viability of HCT116 and A549 cell lines.

Keywords: *Chenopodium foliosum*; antibacterial; antifungal; anticancer

Chenopodium foliosum Metanol Ekstraktının Biyolojik Aktivitelerinin Araştırılması

Özet: *Chenopodium* cinsi Türkiye dahil hemen hemen dünyanın her yerinde yayılış göstermektedir. Bu cinse ait türler farklı ülkelerin geleneksel tıbbında çeşitli hastalıkların tedavisinde kullanılmaktadır. *Chenopodium* cinsinin farklı türleri antikanser, antifungal ve antibakteriyel etkiler göstermektedir. *Chenopodium foliosum (C. foliosum)* ile ilgili literatürde büyük bir boşluk bulunmaktadır. Bu çalışmada *C. folisoum* bitkisinin metanol ekstraktının biyolojik aktiviteleri araştırıldı. Antikanser aktivitesi, HCT116 ve A549 hücre hatlarında 3-(4,5-Dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromür (MTT) analizi kullanılarak değerlendirildi. Antibakteriyel ve antifungal aktivitesi minimum inhibisyon konsantrasyonu (MİK) tahlili ile incelenmiştir. *C. folisoum*'un metanol ekstraktı, HCT116 ve A549 hücre hatlarının hücre canlılığını sırasıyla %73.4 ± 3.3 ve %78.7 ± 2.3'e düşürdüğü saptanmıştır. *C. foliosum* ayrıca *Candida albicans*'ın (*C. albicans*) hücre

canlılığını %91,2'ye düşürdüğü saptanmıştır. Araştırmamız *C. folisoum* metanol ekstraktının HCT116 ve A549 hücre canlılığını kontrole göre azaltığını göstermektedir.

Anahtar Kelimeler: *Chenopodium foliosum*; antibakteriyel; antifungal; antikanser

1. Introduction

Cancer is one of the diseases that threatens life by invading and disrupting the surrounding tissues, along with the irregular proliferation of abnormal cells [1]. Many advances have been made in cancer treatment methods, but the incidence and mortality rates still remain very high [2]. Since most cancer treatment methods are not selective, they cause many side effects. Most cancer patients, on the other hand, turn to complementary medicine (herbs or herbal supplements) to alleviate the effects of cancer treatment [3]. There have been reports of pro-apoptotic and anti-proliferative effects of phytochemicals and antioxidants that are naturally found in plants. Half of the 200 chemical compounds that have been licensed for use in cancer treatment come from natural sources [4].

One of the critical problems for human health is diseases caused by pathogenic microorganisms [5]. In the treatment of pathogenic diseases, medicinal plants have been used by humans since ancient times. Approximately 80% of people in developing countries use medicinal herbs to treat infectious diseases [6]. It has been reported that the roots, stems, leaves, and seeds of various plants have antimicrobial and free radical scavenging abilities, making them useful as both antibiotics and antioxidants [7-8]. A large number of species of bacteria and fungi are known to cause serious human diseases. *Escherichia coli* (*E. coli)* causes harmful diseases in humans, such as endemic problems, intestinal problems [9]. *Pseudomonas aeruginosa (P. aeruginosa)* causes nosocomial infections such as pneumonia [10], urinary tract infections (UTIs) [11], wounds [12], and bloodstream [13]. *Staphylococcus aureus* (*S. aureus)* is one of the most common pathogens worldwide and known to cause a large number of diseases, including moderately severe skin infections, fatal pneumonia and sepsis [14-15]. In recent years, *Candida* species (*Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis* and *Candida krusei*) have been identified as causes of invasive infections and mortality [16-17].

Traditional medicine has employed several *Chenopodium* species to treat a wide range of illnesses. Biological activities of different *Chenopodium* species have been reported, including antipruritic, antinociceptive, antimicrobial, anthelmintic, tumor-inducing, vermifuge, antiviral, hemagglutination", antifungal, immunomodulatory, cytogenetic, cytotoxic, hypotensive and spasmolytic activity [18]. *C. foliosum* has been used in traditional medicine in various countries. It has been used in eye infections and constipation complaints in Pakistan [19], as an immunostimulant and in cancer treatments in Bulgaria [20], and against shortness of breath in Erzurum-Turkey [21].

For these reasons, it was aimed to investigate the anticancer, antibacterial and antifungal properties of the methanol extract of *C. foliosum*. MTT analysis was performed on A549 (human lung adenocarcinoma cell line) and HCT116 (human colorectal carcinoma cell line) cell lines to determine the anticancer activity. MIC analysis was performed on two gram-negative bacteria (*E. coli* and *P. aeru-*

ginosa) and one gram-positive (*S. aureus*) bacterium to determine the antibacterial activity. Additionally, MIC analysis was performed on the *Candida albicans (C. albicans)* and *Candida glabrata* (*C. glabrata*) to determine the antifungal activity.

2. Material and Methods

2.1. Plant materials & preparation of extract

The plant has been systematically collected and described by Pelin Yılmaz SANCAR. Plant samples were collected during the fruiting period [*C. foliosum* (Moench) Aschers., B8-Elazığ: Baskil, Haroğlu mountain, steppes, 1800-1900 m., 03.07.2022]. The leaves and fruit parts of the plant were dried. The powdered plant materials were extracted with methanol for 72 hours and then evaporated in a sterile cabinet. After that, a whatman filter paper was used to filter the plant extracts. The plant extract was dissolved with DMSO [22].

2.2. Anticancer activities

Two cancerous cell lines, A549 and HCT116, were used to determine anticancer properties. All cells were allowed to grow in a DMEM medium supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin at 37°C in an atmosphere of 5% CO_2. The cells were grown in a 96-well microplate at a density of 10^4 cells with a medium volume of 100 ul per well. After 24-hour incubation the cells were washed with 1x PBS and treated with different concentrations of plant extract for 24 hours ranged from 800 µg/mL to 50 µg/mL. MTT (5 mg/mL) reagent was added to each well and incubated for 4 hours. After incubation, DMSO was added to the wells and incubated for 15 minutes. Then, the absorbance was measured using a microplate reader at a wavelength of 570 nm. IC_{50} was calculated based on linear cell viability percentages. The following formula yields the percentage of cell viability [23].

$$Cell\ viability\ (\%) = \frac{OD\ of\ sample\ at\ 570\ nm}{OD\ of\ control\ at\ 570\ nm} x100 \quad (2.1)$$

2.3. Antimicrobial activities

Two gram-negative bacterial species, *E. coli, P. aeruginosa* and one gram-positive *S. aureus* bacterial species were used for antibacterial tests. *C. albicans* and *C. glabrata* fungal species were used for antifungal tests. The BMD (Broth Microdilution) test was utilized for determining the MIC in antifungal [24] and antibacterial analyses [25]. Plant stock solution was diluted in serial dilutions with YPD (Yeast Peptone Dextrose) medium (2% peptone, 2% glucose, 1% yeast extract) for yeasts, LB (Luria-Bertani) broth medium (1% tryptone, 1% NaCl, 0.5% yeast extract) for bacteria and added to 96-well plates. Yeast (1-5×10^5 CFU/mL) and bacterial (1×10^6 CFU/mL) solutions (inoculums) were prepared in sterile water and added in equal volumes to 96-well plates containing different concentrations of plant extract. The plates were incubated at 37°C for 24 hours. Following incubation, the MIC was determined spectrophotometrically at 540 nm for yeasts and analyzed by eye for bacteria. The MIC value was measured as the lowest concentration of drugs that did not cause noticeable growth of

bacteria and as the lowest concentration of drugs that caused at least a 50% or more decrease in growth in yeasts compared to the control (non-drug) group.

2.4. Statistical analysis

A two-tailed student's t-test was used to compare the effects of the applied concentrations with the control group. It was assumed that the data showed a normal distribution and there was a homogeneity of variance between the groups.

3. Results and Discussion

Medicinal plants have been used in traditional health systems since prehistoric times and remain a significant source of health for a large part of the world's population [26]. It has been reported that species belonging to the genus *Chenopodium* are used in traditional treatments and possess various medicinal properties. These plants are employed in the treatment of various diseases, such as chest complaints, coughs, abdominal pain, lung congestion, and nerve disorders [18]. Additionally, pharmaceutical researchers have reported antibacterial, antifungal, and anticancer activities of these plants [27-31]. Phytochemical analyses have been reported in the aerial parts of the *C. foliosum* and compounds found in the methanol extract include carbohydrates, flavonoids, saponins, and alkaloids/amine [32]. Secondary metabolites found in medicinal plants, including terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, and alkaloids, exhibit significant antioxidant activity and are crucial in cancer treatment [33]. Numerous investigations have demonstrated the anti-inflammatory, antitumor, antimutagenic, and anticarcinogenic properties of antioxidant substances [34]. Therefore, medicinal plants play an essential role in combating cancer and addressing current and future health needs. Thanks to the secondary metabolites of medicinal plants, it is possible to prevent diseases such as cancer and treat them with fewer harmful side effects [35].

The anticancer activity of *C. foliosum* methanol extract was tested against two cancer cell lines A549 and HCT116 with 5 increasing concentrations (50-800 µg/mL). Cells treated with the methanol extract for 24 hours were evaluated for cytotoxicity using MTT analysis. It was revealed that *C. foliosum* methanol extract reduced cell viability of the HCT116 to 73.4 ± 3.3 %, 75.6 ± 4.2 %, 81.1 ± 6.2 %, 88.8 ± 7.3 %, and 88.7 ± 9.6 % at 800 µg/mL, 400 µg/mL, 200 µg/mL, 100 µg/mL and 50 µg/mL respectively (Figure 1). A similar trend was observed against A549, as cell viability was reduced to 78.7 ± 2.3 %, 79.8 ± 4.15 %, 91.4 ± 2.8 %, 96.2 ± 1.5 % and 98.4 ± 5.5 % at 800 µg/mL, 400 µg/mL, 200 µg/mL, 100 µg/mL and 50 µg/mL respectively (Figure 1). In addition, IC_{50} values for HCT116 and A549 cell line were found to be 1.494 mg/mL and 1.678 mg/mL, respectively.

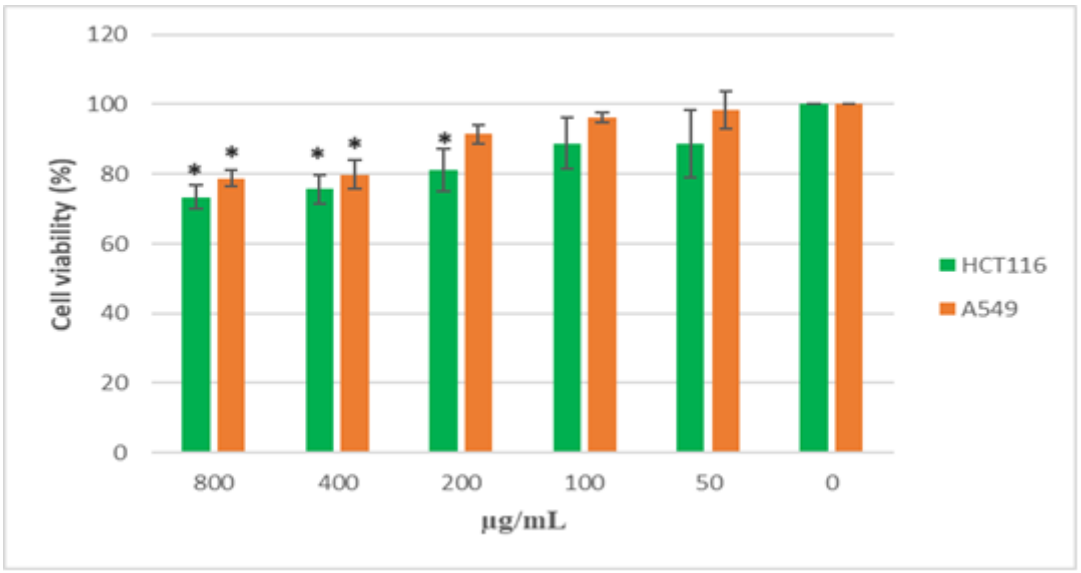

Figure 1. Effect of *C. foliosum* methanol extract on the viability of HCT116 and A549 cell lines based on MTT assay. The results represent mean ± SD. The asterisk mark (*) indicates significant difference ($p<0.05$) when analyzed using Student's t-test.

Data on the cytotoxic and anticancer activities of *Chenopodium* species are limited. According to the literature, methanol extract of *C. bonus-henricus* and some of its compounds have been reported to show cytotoxic effects depending on concentration (IC50 doses ranging from 124.5 to 258 µg/mL) in various cell lines (HL-60, SKW-3, Jurkat E6-1, BV-173 and K-562) [36]. A strong antitumor effect and the potential to prevent cancer formation of *C. ambrosioides L.* have been reported [37-38]. Ascaridole, which has been detected in *C. foliosum* essential oil by GC-MS analysis [39], has been reported to have a cytotoxic effect against various tumor cell lines (CCRF-CEM, HL 60, MDA-MB-231) [40]. It has been reported that 30-normedicagenic acid glycosides isolated from *C. foliosum* show significant cytotoxic activity in BV-173, SKW-3 and HL-60 leukemic cell lines and exhibiting possible immunomodulatory properties by moderately increasing the production of interleukin-2 in PHA/PMA-stimulated Jurkat E6.1 cells [41]. In this study, the cytotoxic effect of *C. foliosum* methanol extract was evaluated in two cancerous cell lines. The current findings showed that *C. foliosum* methanol extract has cytotoxic effects on both A549 and HCT116 cells at 800 and 400 µg/mL. However, its effect remained low compared with previous studies. This discrepancy may be caused by differences between species and also among the same species grown in different areas, as their chemical composition may vary.

Plant extracts have long been used traditionally to treat bacterial, fungal, viral, and other microbiological illnesses [42]. The antimicrobial activities of *C. foliosum* methanol extract were determined according to the BMD procedure. Testing of antibacterial activity was conducted with *E. coli*, *P. aeruginosa*, *S. aureus* and antifungal activity was examined against *C. glabrata* and *C. albicans* with 5 different increasing concentrations (50-800 µg/mL). Effect of the *C. foliosum* on strains treated with the methanol extract for 24 hours was determined by MIC values. Although it was observed that the cell viability of *C. albicans* reduced to 91.2 ± 0.18 %, 93.9 ± 0.21 %, 94.2 ± 0.20 %, 93.7 ± 0.22 %,

and 95.4 ± 0.18 % at 800 µg/mL, 400 µg/mL, 200 µg/mL, 100 µg/mL, and 50 µg/mL, respectively (Figure 2), these decreases were not statistically significant ($p > 0.05$). However, there was no effect on *C. glabrata* (Figure 2).

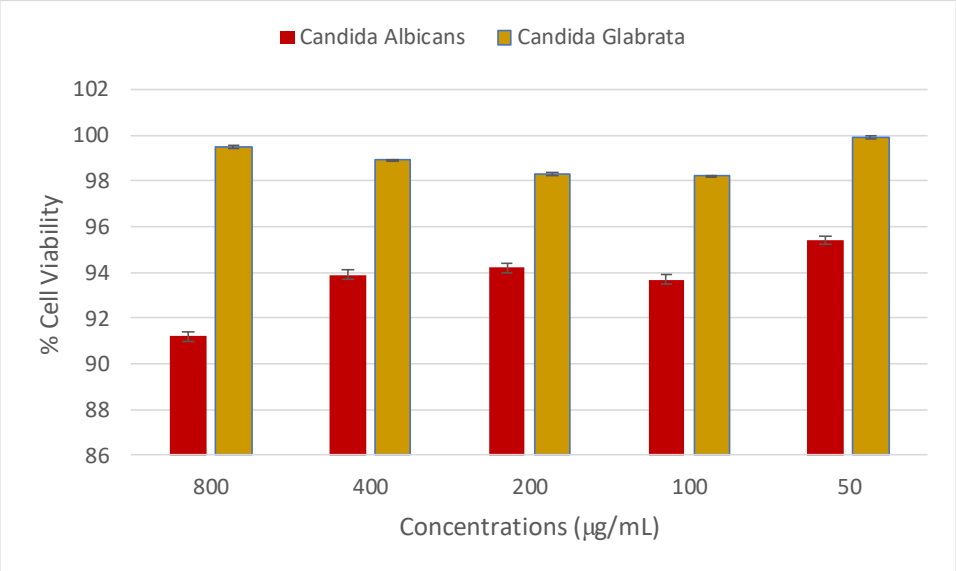

Figure 2. Antifungal effect of *C. foliosum* methanol extract on *C. albicans* and *C. glabrata.* The results represent mean ± SD.

We have also evaluated antibacterial effect of *C. foliosum* methanol extract on *S. aureus, E. Coli* and *P. aeruginosa*. Our result showed that there is no effect on tested bacteria (Table 1).

Table 1. *The effect of C. foliosum methanol extract on S. aureus, E. coli and P. aeruginosa*

Concentration (µg/mL)	**MIC**		
	Gram positive bacteria	**Gram negative bacteria**	
	S. aureus	***E. coli***	***P. aeruginosa***
800	NA	NA	NA
400	NA	NA	NA
200	NA	NA	NA
100	NA	NA	NA
50	NA	NA	NA

(NA: Not active).

According to the literature, aqueous and methanolic extracts of *C. glacum* have been reported as strong antimicrobial agents [43]. Antibacterial properties have been reported in species such as *C. ambrosioides* and *C. album* [44-45], and hexane extracts of *C. ambrosiodes* are reported to inhibit the growth of filamentous fungi [46]. In our research, the antimicrobial activities of the methanol extract were determined by broth microdilution procedure and tested at different concentrations. According to the MIC results, the extract of *C. foliosum* reduced viability on *C. albicans* but did not show any effect on the tested bacteria.

4. Conclusion

In contrast to previous research indicating that the genus *Chenopodium* exhibits strong antibacterial, antifungal, and anticancer effects on various organisms and cell lines, our results showed that *C. foliosum* has a relatively reduced cytotoxic effect against A549 and HCT116 cell lines. These controversial results may be caused by different species used in these experiments as they distributed almost all over the world, including Turkey. This situation may lead different levels and amounts secondary metabolites that show anti-cancer and anti-microbial effects. However, more research is required to determine the precise function of it.

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Conflict of Interest

The authors report no conflict of interest relevant to this article.

Research and Publication Ethics Statement

The authors declare that this study complies with research and publication ethics.

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