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Investigation of Cytotoxic Activity of *Anthriscus nemorosa* (M.Bieb.) Spreng. on Lung Cancer Cells

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ABSTRACT

Medicinal plants are considered an important source of human health due to their therapeutic potential against various diseases including cancer. Cancer is a life-threatening disease characterized by uncontrolled cell growth and abnormal signaling processes. The incidence of cancer in society is increasing day by day. The search for active biological resources is important for the discovery of new anticancer drugs. *Anthriscus nemorosa* (M.Bieb.) Spreng. (Apiaceae) is a medicinal plant naturally distributed in Turkiye and traditionally used as food and against various diseases. This study investigated the methanol extracts of aerial parts and roots of *A. nemorosa* for cytotoxicity on lung cancer (A549) and non-cancerous (L929) cell lines. According to the results, both extracts showed significant dose-dependent cytotoxic effects on lung cancer cells. IC_{50} value was recorded as 8.29 µg/mL in the aerial parts extract. On the other hand, it was recorded as 3.57 µg/mL in the roots extract. Furthermore, the selectivity indexes were calculated as 5.93 and 3.38 for aerial parts and roots extracts, respectively. In light of the findings, it has been concluded that *A. nemorosa* deserves further anticancer research.

Keywords: *Anthriscus nemorosa*, Apiaceae, Lung cancer, Antiproliferative activity.

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Introduction

Plants have had an important place for humans as food and medicine since ancient times. Especially, medicinal plants are considered an important source of human health due to their therapeutic capabilities against various diseases [1]. Identification of cytotoxic natural compounds and medicinal plants will be useful for cancer treatment. The genus *Anthriscus* Pers. (Apiaceae) has 16 species with traditional use worldwide [2]. The genus is represented by 8 species in Turkiye: *A. caucalis* M. Bieb., *A. cerefolium* (L.) Hoffm., *A. kotschyi* Fenzl ex Boiss., *A. lamprocarpa* Boiss., *A. nemorosa* (M.Bieb.) Spreng., *A. ruprechtii* Boiss., *A. sylvestris* (L.) Hoffm., and *A. tenerrima* Boiss. & Spruner [3]. *A. nemorosa* is known as "gimigimi, peçek" and fruits of the plant are used in the treatment of inflammation, gastrointestinal disorders, and rheumatism [4, 5]. *A. nemorosa* is also used as food in the eastern and southeastern parts of Anatolia [6].

There are limited biological activity studies on *A. nemorosa* in the literature. The essential oil of *A. nemorosa* was reported to exhibit antimicrobial activity against *Bacillus subtilis* and *Candida albicans* [7]. In another study, *A. nemorosa* essential oil inhalation was found to improve memory formation and showed anxiolytic and antidepressant effects in treatment groups [2]. Karakaya et al. reported that the essential oil and ethyl acetate fraction of *A. nemorosa* root had high total phenolic content and DPPH radical scavenging activity. The essential oil of the plant also exhibited high butyryl cholinesterase enzyme (BuChE) inhibition in the same study [8]. The essential oil of *A. nemorosa* and farnesene in the chemical composition of the essential oil was reported to be effective against *Trypanosoma brucei* [9]. Forouhandeh et al. investigated the cytotoxic activity of *n*-hexane, dichloromethane, and methanol extracts of *A. nemorosa* on breast cancer cells. *n*-Hexane and 60%, 80%, and 100% fractions of *n*-hexane extract were found to inhibit the proliferation of MCF-7 cells in a dose-dependent manner [10]. *A. sylvestris* and its main compound, anthricin, were found to have strong cytotoxic effects on various cancer cell lines [11-14]. Therefore, *A. nemorosa* may be a promising source against lung cancer due to its phylogenetic relationship.

In this study, the methanol extracts of the roots and aerial parts of *A. nemorosa* were examined on lung cancer and healthy cells using the MTT method. In this way, it was aimed to introduce new natural strategies with cytotoxic effects against lung cancer to the literature.

Materials and Methods

Plant Material

Anthriscus nemorosa (M.Bieb.) Spreng was collected from Karayün, Sivas in May 2023. The plant was identified by Assoc. Prof. Mehmet Ufuk Özbek from Gazi University, Faculty of Science, Department of Biology. A voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Gazi University, Ankara (GUEF No: 3846).

Preparation of the Extracts

The air-dried roots and aerial parts of the plant were separated and ground to powder using a laboratory-type mill. The plant materials were macerated with methanol at room temperature for 24 h. Then, the extracts were filtered and fresh methanol was added to the residue. These extraction processes were repeated three times (24 h x 3). The filtrates were combined and the solvent was removed by rotary evaporation at 40°C. Extracts obtained from the process were held at +4°C until they were used.

Cell Lines and Cell Culture

Adenocarcinomas of human alveolar basal epithelial cell line (A549, ATCC) and healthy mouse fibroblast cell line (L929, ATCC) were used for the cytotoxicity test of extracts. The cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM, Capricorn Scientific) supplemented with 10% fetal bovine serum (FBS, Serana Europe), 1% L-glutamine, and 1% penicillin-streptomycin (Serana Europe). The cells were incubated at 37 °C under 5% CO_2 humidified atmosphere.

Cell Viability Assay

The cytotoxic activity of the extracts and cisplatin were determined by 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MTT assay called the "gold standard" of cell viability tests is based on measuring metabolic activity [15, 16]. The cells reaching 80% confluence were seeded in 96-well plates (6×10^3 cells/well). After 24 h, cells were treated with five concentrations of the extracts (1-100 µg/mL) in DMSO. Cisplatin was used as a positive control and tested cells were also subjected to cisplatin (1-100 µM). The plates were incubated with test materials for 48 h. After treatment, the supernatant was removed and fresh colorless DMEM and 50 µL MTT solution were added to each well. The cells were maintained at 37 °C for 3 h. DMSO was used to dissolve the resulting formazan crystals. The absorbance was measured at 570 nm using a spectrophotometer (SpectraMax i3x; Molecular Devices, San Jose, CA, USA). Each experiment was conducted in triplicates, and cell viability was indicated as a percentage relative to the control (100% of viability). Data were analyzed using GraphPad Software Prism 8.0 (San Diego, CA, USA; demo version). Nonlinear regression analysis (dose-response) was used to determine the IC_{50} values. IC_{50} values were calculated as µg/mL for extracts and as µM for cisplatin.

Selectivity Index

The selectivity index (SI) indicates the selectivity of test materials between cancer and normal cells. SI was calculated by using the formula [(IC_{50} values of non-cancerous cells)/(IC_{50} values of cancerous cells)]. The compounds with SI greater than 1 are more likely to inhibit cancer cells than non-cancerous cells [17]. When the SI value of a compound is greater than 10, it is considered a selective anticancer agent [18].

Results and Discussion

Extract Yields

The extract yields of *A.nemorosa* were given in Table 1.

Table 1. The extract yields of *A.nemorosa*

Extract	Amount (g)	Yield (%, w/w)
A. nemorosa aerial parts	7.621	15.24
A. nemorosa roots	6.328	12.66

Activity Results

The roots and aerial parts of methanol extracts of *A. nemorosa* were studied to find new approaches against lung cancer. According to the National Cancer Institute (NCI), the extracts with an IC_{50} value of less than 20 µg/mL could be considered promising anticancer substances [19]. In this study, *A. nemorosa* exhibited strong anticancer activity on A549 cell lines in a dose-dependent manner. Cell viability results of the extracts and cisplatin on the tested cells are given in Figure 1.

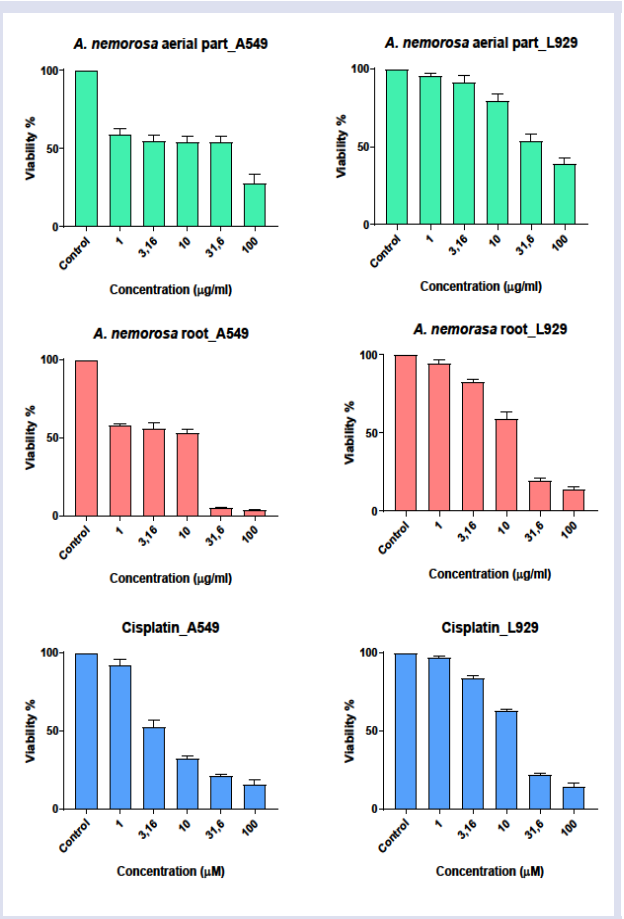

Figure 1. Cell viability inhibition of *A. nemorosa* extracts, and cisplatin against A549, and L929 cell lines after 48 h exposure

Both extracts had an IC_{50} value of ≤ 20 µg/mL against lung cancer cells. IC_{50} values of the aerial parts and roots extracts of *A. nemorosa* on A549 were calculated as 8.29 and 3.57 µg/mL, respectively. It was determined as 49.13 µg/mL for aerial parts extract and 12.07 µg/mL for roots extract on L929. The selectivity of both extracts was higher than cisplatin against normal cells. The cytotoxic effect of *A. nemorosa* extracts and cisplatin against A549, and L929 cell lines were shown in Table 2 and Figure 2.

Table 2. Cytotoxic effect of *A. nemorosa* extracts, and cisplatin against A549, and L929 cell lines after 48 h exposure

	IC_{50} ± SEM (µg/mL)		SI
	A549	L929	
A. nemorosa aerial part	8.29 ± 2.57	49.13 ± 5.58	5.93
A. nemorosa root	3.57 ± 0.57	12.07 ± 0.78	3.38
Cisplatin	4.89 ± 0.61	13.80 ± 0.90	2.82

The results of cisplatin were given as IC_{50} ± SEM (µM). SEM: Standard error of the mean; SI: Selectivity index

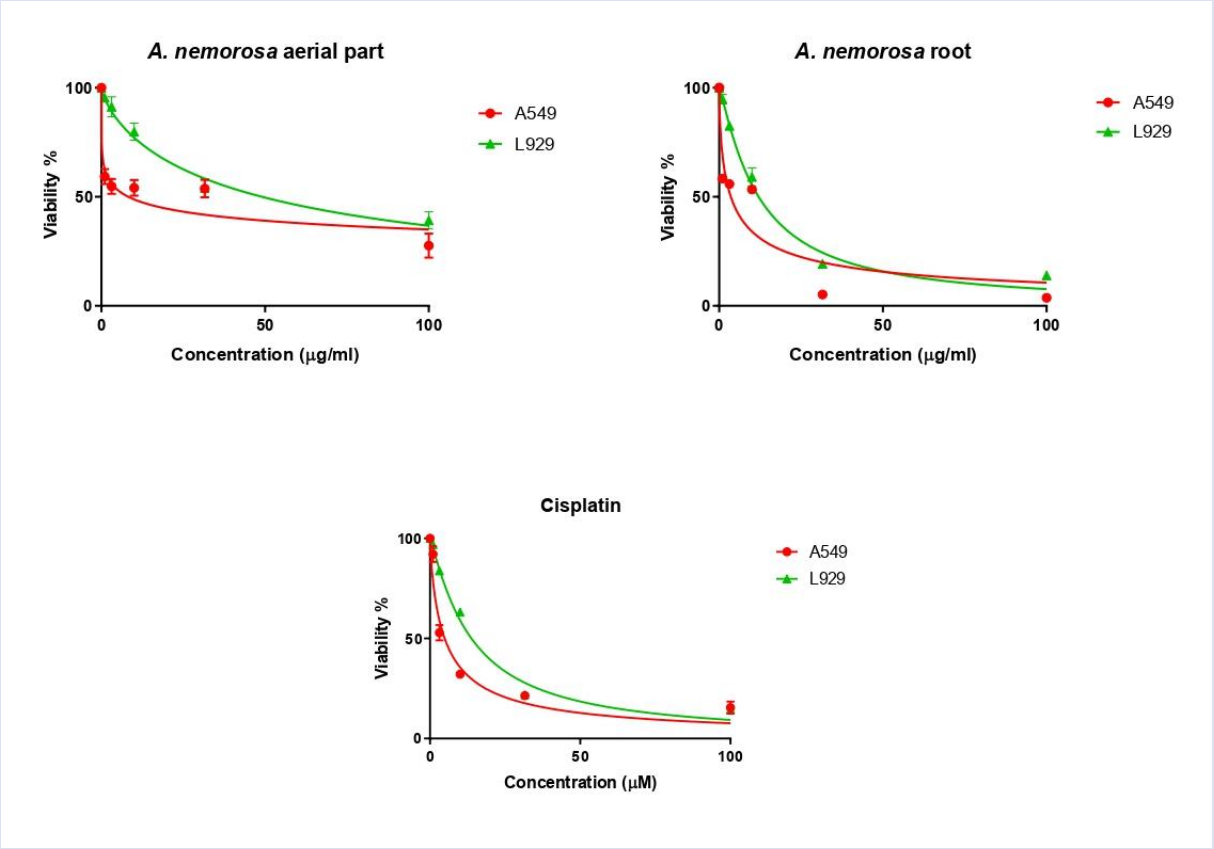

Figure 2. Cytotoxicity effect of *A. nemorosa* extracts, and cisplatin on tested cell lines after 48 h exposure. The plot represents values of mean cell viability % ± SEM

There are a limited number of phytochemical and biological activity studies on *A. nemorosa*. Bagci et al. investigated the effect of *A. nemorosa* essential oil on memory, anxiety, and depression-like behaviors in scopolamine-treated rats. *A. nemorosa* essential oil inhalation improved memory formation and had anxiolytic and antidepressant effects in treatment groups [2]. The root ethyl acetate fraction and essential oil of plant were found to have high total phenolic content with values of 677.31 ve 509.39 GAE g^{-1}. The essential oil of roots showed high butyrylcholinesterase inhibition (88.51%). Major monoterpene of roots and aerial parts of *A. nemorosa* were *α*-pinene (25.5.%), myristicin (10.4%), *p*-cymene (8.2%), limonene (6.0%), and fatty alcohol 1-heptadecanol (7.5%). In another study, the main components of *A. nemorosa* roots essential oil were identified as *n*-nonane (12.1%), *n*-hexadecanol (6.9%), *δ*-cadinene (6.4%), *β*-pinene (6%) and germacrene D (5.4%). In the same study, *A. nemorosa* essential oil was found to be sensitive against *Bacillus subtilis* (MIC= 6.25 µg/mL), and *Candida albicans* (MIC= 50 µg/mL) [7].

There is only one study in the literature on the cytotoxicity evaluation and anticancer potential of *A. nemorosa*. In this study, the cytotoxic effect of *n*-hexane, dichloromethane, and methanol extracts of aerial parts of *A. nemorosa* was examined on breast cancer cells at the concentration range of 50-800 µg/mL. According to the results, *n*-hexane and 60%, 80% and 100% fractions of *n*-hexane extract inhibited the proliferation of MCF-7 cells with IC_{50} values of 75.63, 22.6, 26.82, 14.71, respectively. The predominant escape composition of extracts was identified as non-terpenoid. The most common ingredient was palmitic acid and non-terpenoids were associated with activity against breast cancer cells [10].

Another species of genus *Anthriscus, A. sylvestris,* had cytotoxicity against various cancer cells. The methanol

extract of *A. sylvestris* was reported to have cytotoxicity on human chronic myeloid leukemia K562 cell [14]. Ikeda et al. found that the root and aerial parts of *A. sylvestris* had strong inhibition on the proliferation of gastric adenocarcinoma (MK-1), cervical adenocarcinoma (HeLa), and melanoma (B16F10) cells [13]. The petroleum ether fraction of *A. sylvestris* showed a strong cytotoxic effect on HeLa and human hepatocellular carcinoma (HepG2) cell lines with IC_{50} values of 18.25 and 36.53 µg/mL, respectively [11]. *n*-Hexane and dichloromethane fractions of *A. sylvestris* inhibited the growth of human gastric adenocarcinoma (AGS) cells [12]. Anthricin (deoxypodophyllotoxin) was reported as one of the main lignans in *A. sylvestris* [20]. This compound had various biological activities, including anticancer, and was considered the most important compound of the plant [21-23]. Anthricin had a five-ring cyclolignan structure and similar to podophyllotoxin. Podophyllotoxin served as a key starting compound for the synthesis of etoposide and teniposide, anticancer drugs used in the treatment of various leukemia and solid tumors. Since podophyllotoxin was synthesized directly from deoxypodophyllotoxin in the biosynthetic pathway, anthricin also gained importance as the starting compound in the synthesis of etoposide and teniposide [22, 24]. Anthricin was reported to have a strong antiproliferative effect on HepG2, osteosarcoma (MG63), melanoma (B16), HeLa, and breast cancer cells (MCF7, MDA-MB-231) [11, 12, 21]. It was reported that anthricin inhibited polymerization via binding directly to microtubules, causing cell cycle arrest at the G2/M phase and apoptosis [23].

Essential oil analysis stands out in the literature on *A. nemorosa*. However, in a study, two lignan lactones namely savinin and nemerosin were isolated from methanol extract of *A. nemorosa* root [25]. Lignan-type compounds such as savinin and nemerosin might be responsible for anticancer activity. Detailed and further phytochemical studies are needed.

Conclusion

The methanol extracts of the roots and aerial parts of *A. nemorosa* showed significant dose-dependent cytotoxic effects on lung cancer cells. IC_{50} value was recorded as 8.29 µg/mL in the aerial parts extract and it was recorded as 3.57 µg/mL in the roots extract. Furthermore, the selectivity indexes were calculated as 5.93 and 3.38 for aerial parts and roots extracts, respectively. According to the NCI guidelines, the methanol extract of *A. nemorosa* exhibited promising antiproliferative activity on lung cancer cells. Moreover, the extracts had lower cytotoxicity against normal cells. The selective and potent activity results of *A. nemorosa* indicate that this compound deserves further anticancer research.

Considering our results, *A. nemorosa* could be determined as a promising candidate for cancer treatment. In further studies, it is recommended to discover cytotoxic compounds against lung cancer by activity-guided isolation studies on *A. nemorosa*.

Conflicts of interest

The author declares no conflict of interest.

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