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Research Article

Selective cytotoxicity of *Arctium* extracts in pancreatic, bladder, and colorectal cancer cell lines: A comparative dose-response study

Tuba Özdemir Sancı[1*], Beyza Ecem Öz Bedir[2], Emine Terzi[2], Benan Kalaycı[3], Tuba Aydın[3], Fevzi Özgökce[4], Ahmet Çakır[5]

[1]Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Türkiye
[2]Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Medical Biology, Ankara, Türkiye
[3]Ağrı Ibrahim Cecen University, Faculty of Pharmacy, Department of Pharmacognosy, Ağrı, Türkiye
[4]Van Yüzüncü Yıl University, Faculty of Science, Department of Molecular Biology and Genetics, Van, Türkiye
[5]Kilis 7 Aralik University, Faculty of Science, Department of Chemistry, Kilis, Türkiye

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Abstract: Gastrointestinal and urinary tract cancers, including colorectal, pancreatic, and bladder malignancies, represent a major global health burden due to their high mortality and therapeutic resistance. In light of the limitations of current treatment strategies, plant-derived compounds with multitargeted actions have emerged as promising alternatives. This study aimed to evaluate the cytotoxic effects of methanol and dichloromethane:methanol (1:1) extracts of *Arctium minus* subsp. *pubens*, A. *minus* subsp. *minus*, and A. *tomentosum* var. *glabrum* on three human cancer cell lines: HTB-9 (bladder), DLD-1 (colorectal), and PANC-1 (pancreatic). Extracts were prepared from aerial parts of plants using two solvents of differing polarity. Cell viability was determined after 24 and 48 hours using the WST-1 assay. IC_{50} values were calculated, and statistical comparisons were performed. All extracts exhibited dose-dependent cytotoxicity. The methanol extract of A. *tomentosum* showed the strongest effect in HTB-9 cells, while A. *minus* subsp. *pubens* demonstrated potent activity against DLD-1 and PANC-1 cells. The findings highlight the selective cytotoxic potential of Arctium species, especially A. *minus* subsp. *pubens* and A. *tomentosum*, which may serve as valuable candidates for further phytochemical and mechanistic anticancer studies.

INTRODUCTION

Cancer remains a leading cause of global mortality, with gastrointestinal and urinary tract malignancies posing significant therapeutic challenges due to their aggressive phenotypes and frequent resistance to chemotherapy. A major number of cancer-related deaths worldwide are associated with malignant tumors of gastrointestinal and urinary tract especially colon, pancreas and bladder (Sung *et al*., 2021; Zhou *et al*., 2024). Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer death in the world. Colorectal cancers, which include colon and rectal cancers, have been steadily decreasing due

*Corresponding Author: Tuba ÖZDEMİR SANCI ozdemirsanci.tuba@aybu.edu.tr Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Türkiye

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to increased cancer screening and better treatment methods (Menon & Cagir, 2025, Nakkaş *et al.*,2023). Pancreatic cancer occurs when pancreatic cells grow and divide uncontrollably,

causing tumors to form. Pancreatic cancer is a persistent malignancy and is the seventh leading cause of cancer deaths globally in industrialized countries. Although advances in diagnostic approaches and systemic treatments have increased over the last decade, it remains a devastating malignancy due to limited options for effective treatment (Hu *et al*., 2021; Mizrahi *et al.*, 2020; Rawla *et al.*, 2019). Bladder cancer is one of the ten most common cancers diagnosed worldwide, accounting for 2.1% of all cancer deaths. More than 70% of patients experience recurrence after initial treatment. There is a fundamental need for non-invasive, sensitive, rapid, and inexpensive biomarkers for the treatment of bladder cancer (Ferro *et al*., 2021; Kubrak *et al*., 2022; Ozdemir-Sancı *et al*.,2024, Terzi *et al.*,2025).

The limitations of existing therapeutic modalities for these malignancies have become increasingly apparent. Conventional chemotherapy approaches often demonstrate narrow therapeutic windows, severe side effects, and transient efficacy due to rapid development of resistance (Rawla *et al*., 2019). While molecularly targeted therapies represent a significant advancement, their utility remains constrained by high costs, variable patient responses, and the inevitable emergence of resistance mechanisms (Atanasov *et al*., 2021). These challenges have stimulated renewed interest in plant-derived compounds as potential sources of novel anticancer agents that may overcome these limitations through their characteristic multi-target mechanisms and more favorable toxicity profiles.

Among botanical candidates, the genus *Arctium* (commonly known as burdock or 'dulavrat otu' in Turkish) has emerged as a particularly promising source of bioactive compounds with demonstrated anticancer potential. While *Arctium lappa* has been the subject of numerous pharmacological investigations, its close relatives *A. minus* (particularly subspecies pubens and minus) and *A. tomentosum* var. *glabrum* have been largely overlooked by the scientific community despite their extensive ethnomedicinal use (Erdemoglu *et al*.,2009, Wang *et al*., 2019). Historical records from Traditional Chinese Medicine and European folk medicine document the employment of these species for treating various neoplastic conditions, with particular emphasis on gastrointestinal and urinary tract malignancies (Su *et al*., 2015; Zhao *et al*., 2009). Contemporary phytochemical analyses have identified over 200 bioactive constituents in *Arctium* species, with lignans (particularly arctigenin and its derivatives), flavonoids, and phenolic acids being the most abundant and biologically active components (de Souza *et al*., 2019; Petkova *et al*., 2021). These compounds have demonstrated diverse anticancer mechanisms in preclinical models, including induction of apoptosis through both intrinsic and extrinsic pathways, inhibition of angiogenesis, suppression of metastatic potential, and modulation of key oncogenic signaling pathways such as STAT3 and NF-κB (Feng *et al*., 2017; Lee *et al*., 2020).

The current investigation represents the first comprehensive evaluation of the cytotoxic potential of *A. minus subsp. pubens*, *A. minus subsp. minus*, and *A. tomentosum* var. *glabrum* against three clinically relevant cancer cell lines: pancreatic (PANC-1), bladder (HTB-9), and colorectal (DLD-1) adenocarcinoma models. Recognizing that extraction methodology critically influences the phytochemical profile and consequent biological activity of plant extracts, we employed a systematic solvent strategy using methanol and dichloromethane:methanol (1:1) to ensure broad-spectrum recovery of both polar and mid-polar bioactive constituents (İlgün *et al*., 2022). The primary objective was to evaluate the time- and dose-dependent effects of these extracts on cell viability in colon, pancreatic, and bladder cancer cells. By integrating traditional knowledge with modern experimental approaches, this study aims to provide foundational insight into the bioactivity of *Arctium* species and support future research into their phytochemical profiles and mechanisms of action.

METHOD

Plant Material and Extraction

Three *Arctium* species, known to be effective in the treatment of stomach cancer, were used as plant material. These species are *Arctium tomentosum* Mill. var. *glabrum* (Körnicke) Arenes (aboveground parts), *Arctium minus* (Hill) Bernh. subsp. *pubens* (Babington) Arenes (belowground parts), and *Arctium minus* subsp. *minus* (aboveground parts). The species identification of the plants collected by Prof. Dr. Fevzi ÖZGÖKÇE in June, July and August 2022 was made and the plant samples were recorded in the Van Yüzüncü Yıl University Herbarium. The plant samples were collected in small quantities to make a herbarium record and to be used in preliminary studies. The region, date, and herbarium codes of the plants are as follows:

Arctium tomentosum Mill. var. *glabrum* (Körnicke) Arenes: Turkiye, A9 Ardahan: Central district, Lehimli Village, 13.08.2022, F 15541, VANF 165257.

Arctium minus subsp. *pubens*: Turkiye, A9 Ardahan: Central district, Çataldere Village, 01.07.2022, F 15542, VANF 165258.

Arctium minus subsp. *minus*: Turkiye, A9 Ardahan: Çıldır district, Baltalı Village, 29.06.2022, F 15543, VANF 165259.

The plant material was shade-dried, powdered, and subjected to sequential maceration (24 h×3) with methanol (MeOH) and a methanol-dichloromethane mixture (MeOH-CH_2Cl_2, 1:1 v/v). The extracts were concentrated under reduced pressure at 45°C, yielding dark-green residues (MeOH: 12.4% w/w; MeOH-CH_2Cl_2: 9.8% w/w), which were stored at −20°C until use.

Cell Culture and Treatments

Human cancer cell lines; DLD-1 (colon adenocarcinoma), Panc-1 (pancreatic carcinoma), HTB-9 (bladder transitional cell carcinoma) were obtained from ATCC. Cells were maintained in RPMI-1640 (DLD-1, HTB-9) or DMEM (Panc-1) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C in a 5% CO_2 humidified incubator. For cytotoxicity assays, cells were seeded at 5×10^3 cells/well in 96-well plates and allowed to adhere for 24 hours. Extracts were dissolved in DMSO (final concentration ≤ 0.1%) and diluted in culture medium to final concentrations of 0, 125, 250, 375, and 500 µg/mL. Controls included untreated cells and vehicle (0.1% DMSO).

Assessment of Cell Viability and IC_{50} Determination

Cell viability was quantified using the WST-1 assay (Roche Diagnostics) after 24 h of treatment. Briefly, 10 µL of WST-1 reagent was added to each well, and plates were incubated for 4 h at 37°C. Absorbance was measured at 450 nm using a microplate reader (Thermo Varioskan).

Statistical Analysis

Data are presented as mean ± standard deviation (SD) from three independent experiments (n=3). Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 9.1.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Cytotoxic Potential of *Arctium* Extracts

In this study, the cytotoxic effects of dichloromethane:methanol (1:1) and methanol extracts obtained from *A. minus* subsp. *pubens*, *A. minus* subsp. *minus* and *A. tomentosum* var. *glabrum* species on HTB-9 (bladder), DLD1 (colorectal) and PANC-1 (pancreatic) cancer cell lines were

evaluated. In the evaluations made by considering cell viability rates and IC_{50} values, it was observed that cytotoxic activity increased in a dose-dependent manner in all extracts.

In HTB-9 bladder cancer cells, the cytotoxic activities of the extracts varied significantly depending on both the plant species and the extraction solvent. After 24 hours of treatment, the methanol extract of *A. tomentosum* var. *glabrum* exhibited moderate activity (IC_{50}: 142.8 ± 3.2 µg/mL), representing the most potent cytotoxic response among all tested extracts at this time point. This suggests that *A. tomentosum* var. *glabrum* may contain fast-acting polar compounds with selective activity against bladder cancer cells, warranting further phytochemical and mechanistic investigation (Table 1).

In the DLD-1 colorectal cancer cell line, both methanol and dichloromethane:methanol extracts of *A. minus* subsp. *pubens* demonstrated the most potent cytotoxic activity at both 24 and 48 hours. At 24 hours, the $CHCl_2$:MeOH extract of this species had an IC_{50} of 105.4 ± 22.1 µg/mL, which further decreased to 69.1 ± 5.2 µg/mL at 48 hours. Likewise, the methanol extract showed enhanced activity at 48 hours (IC_{50}: 52.3 ± 1.8 µg/mL) compared to 24 hours (IC_{50}: 92.4 ± 18.1 µg/mL). *A. tomentosum* var. *glabrum* ($CHCl_2$:MeOH) extract also displayed relatively strong cytotoxicity at 48 hours (IC_{50}: 9 ± 2.5 µg/mL), suggesting remarkable sensitivity of DLD-1 cells to this extract despite its weaker performance at 24 hours. These findings indicate that DLD-1 cells were the most sensitive among the tested cancer cell lines, especially to the $CHCl_2$:MeOH extracts of both *A. tomentosum* and *A. minus* subsp. *pubens* (Tables 1, and 2).

Table 1. IC_{50} values (µg/mL) of different *Arctium* species extracts against HTB-9, DLD-1, and PANC-1 cancer cell lines after 24 hours of treatment.

Extract/Cell Line	*A. tomentosum*-$CHCL_2$:MeOH	*A. tomentosum*-MeOH	*A.minus* subsp.*minus*-$CHCL_2$:MeOH	*A.minus* subsp.*minus*-MeOH	*A.minus* subsp. *pubens*-$CHCl_2$:MeOH	*A.minus* subsp. *pubens* -MeOH
HTB-9	> 500	142±8.2	> 500	438.5±13.6	321.1±23.19	451.2±41.59
DLD-1	171.3±15.59	301.4±8.5	179.4±12.65	219.3±7.6	105.4±21	92.46±18.81
PANC-1	148.3±11.02	173.5±13.06	138.9±21	167.5±10.61	90.35±9.81	101.5±9.43

This table presents the half-maximal inhibitory concentration (IC_{50}) values of methanol (MeOH) and dichloromethane:methanol ($CHCl_2$:MeOH, 1:1) extracts obtained from *Arctium tomentosum*, *A. minus* subsp. *minus*, and *A. minus* subsp. *pubens*. The cytotoxic effects were evaluated in three human cancer cell lines: HTB-9 (bladder), DLD-1 (colorectal), and PANC-1 (pancreatic) following 24 hours of incubation. Values are expressed in micrograms per milliliter (µg/mL) as mean ± standard deviation. ">500" indicates IC_{50} values exceeding the tested concentration range.

In PANC-1 pancreatic cancer cells, *A. minus* subsp. *pubens* extracts again exhibited strong time-dependent cytotoxic effects. At 24 hours, the $CHCl_2$:MeOH extract of this species showed an IC_{50} of 90.3 ± 5.8 µg/mL, which was further reduced to 34.2 ± 1.02 µg/mL at 48 hours, making it the most potent extract in this cell line. The methanol extract of the same species also displayed a significant improvement over time, with IC_{50} values decreasing from 101.5 ± 9.43 µg/mL to 62.5 ± 0.5 µg/mL. Meanwhile, *A. tomentosum* extracts exhibited moderate cytotoxicity at both time points, with methanol and $CHCl_2$:MeOH extracts reaching IC_{50} values of 173.5 ± 13.06 µg/mL and 148.3 ± 11.02 µg/mL at 24 hours, and 143.5 ± 6.3 µg/mL and 109.8 ± 5.6 µg/mL at 48 hours, respectively. The data confirm that *A. minus* subsp. *pubens* $CHCl_2$:MeOH extract exerts the most effective cytotoxic response in PANC-1 cells, particularly over longer exposure times (Tables 1, and 2).

Since the extracts exhibited cytotoxic activity at 24 hours, their effects on cell viability at this time point were evaluated for each cell line. For the HTB-9 bladder cancer cell line, the methanol extract of *A. tomentosum* var. *glabrum* showed the most pronounced cytotoxic effect among all tested samples, causing a steep and concentration-dependent decline in cell viability.

Table 2. IC_{50} values (µg/mL) of different *Arctium* species extracts against HTB-9, DLD-1, and PANC-1 cancer cell lines after 48 hours of treatment.

Extract/Cell Line	A. *tomentosum*-$CHCl_2$:MeOH	A. *tomentosum*-MeOH	A.*minus* subsp.*minus*-$CHCl_2$:MeOH	A.*minus* subsp.*minus*-MeOH	A.*minus* subsp. *pubens*-$CHCl_2$:MeOH	A.*minus* subsp. *pubens*-MeOH
HTB-9	273.2±8.5	113.8±3.6	243.8±10.01	311.2±21.2	168±3.4	210.3±4.8
DLD-1	9±2.5	225.2±7.8	99.92±3.2	149.2±4.01	69.12±1.5	52.32±1.8
PANC-1	109.8±5.6	143.5±6.3	100.6±7.5	120.0±8.2	34.26±1.02	62.50±2.5

This table presents the half-maximal inhibitory concentration (IC_{50}) values of methanol (MeOH) and dichloromethane:methanol ($CHCl_2$:MeOH, 1:1) extracts obtained from *Arctium tomentosum*, *A. minus* subsp. *minus*, and *A. minus* subsp. *pubens*. The cytotoxic effects were evaluated in three human cancer cell lines: HTB-9, DLD-1, and PANC-1 following 48 hours of incubation. Values are expressed in micrograms per milliliter (µg/mL) as mean ± standard deviation.

At the highest dose (500 µg/mL), cell viability dropped below 5%, indicating strong antiproliferative activity. Statistical analysis confirmed that the differences in viability between all tested concentrations were highly significant ($p<0.001$), while comparisons between 250 vs. 375 µg/mL ($p<0.001$) and 375 vs. 500 µg/mL($p<0.001$) concentrations remained significant. These findings highlight both the potency and the concentration sensitivity of this extract, suggesting the presence of active phytochemicals with strong affinity for bladder cancer cells. The sharp decline in viability at mid-to-high concentrations also points to a possible therapeutic threshold that could be optimized for future studies (Figure 1).

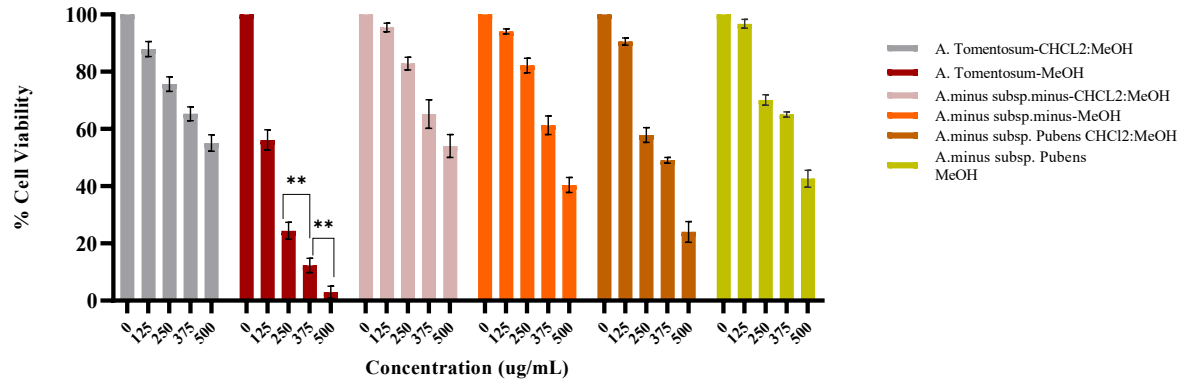


Figure 1. Effect of *Arctium* species extracts on the viability of HTB-9 bladder cancer cells at 24 hours. Cells were treated with increasing concentrations (125, 250, 375, and 500 µg/mL) of dichloromethane:methanol ($CHCl_2$:MeOH, 1:1) and methanol (MeOH) extracts of *A. tomentosum* var. *glabrum*, *A. minus* subsp. *minus*, and *A. minus* subsp. *pubens*.

Since the extracts exhibited cytotoxic activity at 24 hours, their effects on cell viability at this time point were evaluated for the DLD-1 colorectal cancer cell line. Among the tested samples, the dichloromethane:methanol ($CHCl_2$:MeOH) extract of *A. tomentosum* var. *glabrum* demonstrated the most pronounced cytotoxic effect, significantly reducing cell viability in a concentration-dependent manner. At the highest dose (500 µg/mL), viability dropped below 25%, with statistically significant differences observed between all applied concentrations. This suggests a strong antiproliferative effect of this extract on colorectal cancer cells. Similarly, both $CHCl_2$:MeOH and MeOH extracts of *A. minus* subsp. *pubens* also caused notable reductions in viability, with statistical significance between specific concentrations. These results indicate that DLD-1 cells are particularly sensitive to these extracts and that their bioactivity is likely influenced by both the chemical composition of the plant species and the polarity of the extraction solvent (Figure 2).

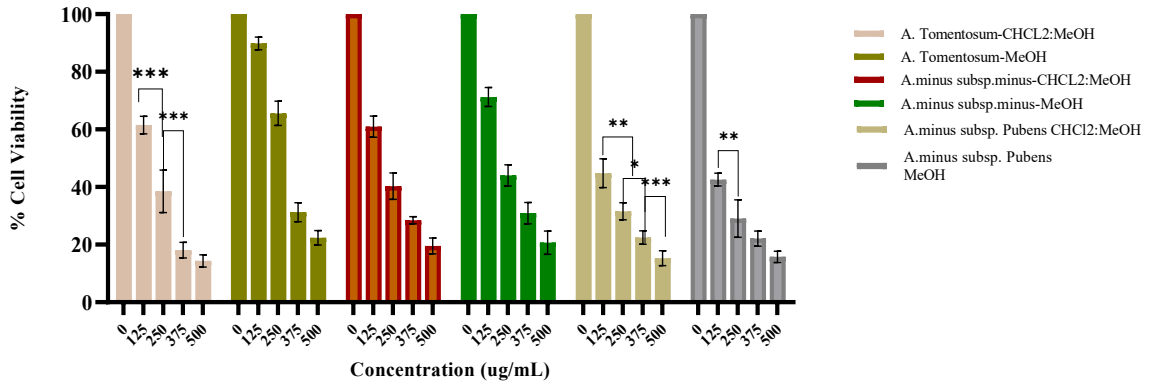


Figure 2. Effect of *Arctium* species extracts on the viability of DLD-1 colorectal cancer cells at 24 hours. Cells were exposed to increasing concentrations (125, 250, 375, and 500 µg/mL) of dichloromethane:methanol ($CHCl_2$:MeOH, 1:1) and methanol (MeOH) extracts from *A. tomentosum* var. *glabrum*, *A. minus* subsp. *minus*, and *A. minus* subsp. *pubens*.

Cell viability was assessed using the WST-1 assay and is expressed as a percentage relative to untreated controls. For PANC-1 pancreatic cancer cell line, the $CHCl_2$:MeOH extract of *A. tomentosum* exhibited the highest cytotoxic effect, with a sharp dose-dependent decrease in viability, reaching below 25% at the highest concentration. Statistical analysis showed highly significant differences between all concentrations (p<0.001), and additional significance between 125/250 and 250/375 µg/mL (p <0.001, indicated by ***). Both $CHCl_2$:MeOH and MeOH extracts of *A. minus* subsp. *pubens* also demonstrated notable cytotoxic effects, with statistical significance ranging from p<0.05 (indicated by *) to p<0.01 (indicated by **). Data represent mean ± SD of three independent experiments (Figure 3).

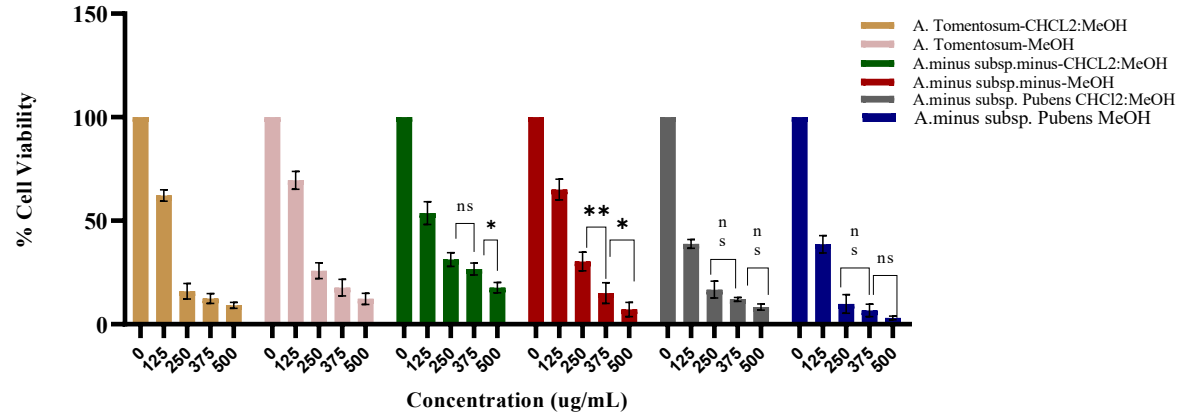


Figure 3. Effect of *Arctium* species extracts on the viability of PANC-1 pancreatic cancer cells at 24 hours. Cells were treated with increasing concentrations (125, 250, 375, and 500 µg/mL) of dichloromethane:methanol ($CHCl_2$:MeOH, 1:1) and methanol (MeOH) extracts derived from *A. tomentosum* var. *glabrum*, *A. minus* subsp. *minus*, and *A. minus* subsp. *pubens*.

A statistically significant reduction in viability was observed for the $CHCl_2$:MeOH extract of *A. minus* subsp. *minus* between 250 and 375 µg/mL (p<0.01 indicated by **). Both extracts of *A. minus* subsp. *pubens* showed strong reductions in viability across all concentrations tested; however, no significant differences were observed between adjacent doses (ns). Data are presented as mean ± SD from three independent experiments.

Collectively, the findings of this study demonstrate that all tested extracts exhibited dose- and time-dependent cytotoxic effects across the HTB-9, DLD-1, and PANC-1 cancer cell lines, with variations in potency depending on the species and extraction solvent. *A. tomentosum* var. *glabrum* methanol extract showed the most prominent activity in HTB-9 bladder cancer cells at 24 hours, significantly reducing cell viability below 5% at the highest concentration, with

strong statistical significance across doses. In DLD-1 colorectal cancer cells, both $CHCl_2$:MeOH and MeOH extracts of *A. minus* subsp. *pubens* displayed substantial cytotoxicity at 24 and 48 hours, while *A. tomentosum* ($CHCl_2$:MeOH) extract was particularly effective at 48 hours, showing the lowest IC_{50} value among all tested combinations. In PANC-1 pancreatic cancer cells, *A. minus* subsp. *pubens* $CHCl_2$:MeOH extract was the most potent, with markedly reduced IC_{50} values over time. Although several extracts significantly reduced cell viability in PANC-1 cells, statistically significant differences between concentrations were limited, suggesting rapid saturation of cytotoxic effects. Overall, the data indicate that *A. minus* subsp. *pubens* and *A. tomentosum* var. *glabrum* contain promising bioactive compounds with selective cytotoxic potential, and their efficacy appears to be influenced by both solvent polarity and cancer cell type. Among all extracts, the methanol extract of *A. tomentosum* induced the most significant reduction in viability in a dose-dependent manner. Statistical analysis revealed a highly significant difference between all concentration groups ($p<0.001$), with additional significant differences observed between 250 vs. 375 µg/mL and 375 vs. 500 µg/mL concentrations ($p<0.01$, indicated by **). Data are presented as mean ± SD of three independent experiments.

DISCUSSION and CONCLUSION

This study provides the first comprehensive evaluation of the cytotoxic effects of *A. minus* subspecies (*pubens* and *minus*) and *A. tomentosum* var. *glabrum* against pancreatic (PANC-1), bladder (HTB-9), and colorectal (DLD-1) cancer cell lines, revealing significant dose-dependent cytotoxicity and notable selectivity among species-cell line combinations. While previous research has predominantly focused on *A. lappa*—demonstrating its broad pharmacological properties including antiproliferative (Predes *et al.*, 2011), hepatoprotective (El-Kott & Bin-Meferij, 2015), antidiabetic (Jianfeng Cao, 2012), anti-inflammatory/analgesic (Conea *et al*., 2017), gastroprotective (dos Santos *et al*., 2008), and antioxidant effects (Skowrońska *et al*., 2021)—our findings highlight that these lesser-studied *Arctium* taxa exhibit comparable or superior cytotoxic activity against gastrointestinal and urinary tract cancers. Notably, *A. minus* subsp. *pubens* methanol extract showed exceptional potency against PANC-1 cells (IC_{50}: 34.2 µg/mL), outperforming reported values for *A. lappa* in similar models. The superior efficacy of methanol extracts in our study contrasts with some *A. lappa* studies where less polar solvents demonstrated better activity, underscoring the need for species-specific optimization of extraction protocols.

The solvent-dependent effects observed in our study warrant particular attention. Methanol extracts consistently demonstrated stronger cytotoxic activity than dichloromethane:methanol (1:1) extracts across all tested cell lines. This finding aligns with work by de Souza et al. (2019), who noted that methanol more efficiently extracts polar cytotoxic compounds like lignan glycosides from *Arctium* species. However, our results contrast with some reports on *A. lappa*, where less polar solvents sometimes showed better activity (Predes *et al*., 2011). This discrepancy may reflect fundamental phytochemical differences between species, emphasizing the need for standardized extraction protocols when comparing *Arctium* taxa.

The time-dependent increase in cytotoxicity we observed (e.g., decreasing IC_{50} values from 24 to 48 hours) suggests cumulative mechanisms of action. This pattern mirrors findings by Lu et al. (2015), who demonstrated that arctigenin from *A. lappa* required prolonged exposure to induce apoptosis in hepatocellular carcinoma cells. Our results extend this observation to novel *Arctium* species and additional cancer types, supporting the hypothesis that certain bioactive components may need time for metabolic activation or gradual disruption of multiple cellular targets. The particularly strong response of DLD-1 colorectal cells to *A. tomentosum* extracts (IC_{50}: 9 µg/mL at 48 h) may reflect enhanced uptake or activation pathways in this cell type, possibly related to their specific molecular characteristics.

Extensive research has identified multiple bioactive compounds in *Arctium* species with significant anticancer potential. *A. lappa* roots contain water-soluble polysaccharides (ALP-1) with anti-inflammatory properties (Zhang *et al*., 2019) and sitosterol-β-D-glucopyranoside showing α-glucosidase inhibitory activity (Miyazawa *et al*., 2005). Various extracts demonstrate notable cytotoxicity: dichloromethane extracts of *A. minus* exhibit potent activity against MDA-MB-231 breast cancer cells (IC_{50} 10.8-21.4 μg/mL) (İlgün *et al*., 2022), while its methanolic extract shows effectiveness against MCF-7 and MDA-MB-231 lines (IC_{50} 0.97-1.26 mg/mL) (Şakul *et al*., 2022). *A. lappa* extracts display broad-spectrum activity, with n-hexane extracts showing particular efficacy against MCF-7 cells (IC_{50} 14.1 μg/mL) (Taleb Agha *et al*., 2020) and ethanol extracts varying effects across gastrointestinal cancers (IC_{50} 10-2030 μg/mL) (Naghavi Alhosseini *et al*., 2021). Isolated compounds reveal remarkable specificity - lignans (lappaols A/C/F) demonstrate prostate cancer activity (IC_{50} 8-40 μg/mL) (Ming *et al.,* 2004) with lappaol F showing 54% tumor growth inhibition *in vivo* (Sun et al., 2014), while arctigenin exhibits exceptional potency against leukemia (IC_{50} 1 μM) (Matsumoto *et al.,* 2006) and enhances doxorubicin efficacy by suppressing MRP-1 expression (Lee *et al*., 2020). The compound onopordopicrin further shows promise against colorectal carcinoma (IC_{50} 19.7 μg/mL) (Machado *et al*., 2012). These findings collectively underscore the diverse pharmacological potential of Arctium species through both crude extracts and purified compounds.

The findings of this study are in line with previous research that reported the antiproliferative and cytotoxic effects of *Arctium* species in various cancer models. However, our study extends the current knowledge by specifically highlighting the strong cytotoxic potential of *A. minus* subsp. *pubens* and *A. tomentosum* var. *glabrum*, which have been less explored compared to *A. lappa*. Notably, the $CHCl_2$:MeOH and MeOH extracts of *A. minus* subsp. *pubens* showed potent activity against colorectal and pancreatic cancer cell lines, comparable to or even exceeding some of the effects reported for *A. lappa* in breast, hepatic, and gastrointestinal models. Likewise, *A. tomentosum* var. *glabrum* exhibited a remarkable cytotoxic response in HTB-9 bladder cancer cells, suggesting potential tissue-specific selectivity. Unlike studies focusing on purified compounds (e.g., arctigenin with IC_{50} values in the micromolar range), our investigation highlights the therapeutic potential of crude extracts, which may better mimic traditional preparations and offer synergistic benefits from multiple phytochemicals.

The clinical implications of these findings are particularly significant for pancreatic and bladder cancers, which remain challenging to treat. The selective cytotoxicity observed against these malignancies, combined with the relatively low IC_{50} values (all <100 μg/mL), suggests these extracts may target pathways distinct from conventional chemotherapeutics. This is supported by emerging evidence that *Arctium* compounds can overcome multidrug resistance mechanisms (Lee *et al.,* 2020), though the specific pathways involved in our models require further investigation. This study has several important limitations that should be considered. The use of extracts, while ecologically relevant, precludes identification of specific active compounds. Additionally, the in vitro model system cannot fully replicate tumor microenvironment complexities. Future studies should focus on detailed mechanistic studies of cell death pathways and *in vivo* validation using appropriate animal models. Standardization of extraction protocols will be particularly important given the observed species- and solvent-specific variations in bioactivity.

In conclusion, this work establishes *A. minus subspecies* and *A. tomentosum* var. *glabrum* as promising sources of novel anticancer agents, particularly for gastrointestinal and urinary tract malignancies. The robust dose-response relationships and tissue selectivity patterns demonstrated here provide a strong foundation for future phytochemical and pharmacological investigation, while validating traditional ethnomedicinal uses of these plants. Further research should focus on characterizing the active constituents and elucidating their mechanisms of action to facilitate potential clinical translation.

Acknowledgments

Acknowledgments of people, grants, and funds should be placed in a separate section before the References. If the study has been previously presented at a conference or a scholarly meeting, it should be mentioned here.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent with e-maill upon request.

Authorship Contribution Statement

Tuba Özdemir Sancı: Investigation, Methodology, Supervision and Writing-original draft, **Beyza Ecem Öz Bedir:** Software, Formal Analysis, and Writing-original draft, **Emine Terzi:** Software, Formal Analysis, and Writing-original draft, **Benan Kalaycı:** Methodology, Validation, **Tuba Aydın:** Methodology, Validation, Writing-original draft, **Fevzi Özgökçe:** Resources, Visualization, **Ahmet Çakır:** Investigation, Methodology, Writing-original draft.

Orcid

Tuba Özdemir Sancı https://orcid.org/0000-0002-9468-4719
Beyza Ecem Öz Bedir https://orcid.org/0000-0002-0596-834X
Emine Terzi https://orcid.org/0000-0001-9106-3848
Benan Kalaycı https://orcid.org/0000-0003-3248-2377
Tuba Aydın https://orcid.org/0000-0002-7653-6480
Fevzi Özgökçe https://orcid.org/0000-0002-3119-8561
Ahmet Çakır https://orcid.org/0000-0003-1672-1438

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