Adıyaman Üniversitesi Sağlık Bilimleri Dergisi, 2025;11(3):205-214
doi:10.30569.adiyamansaglik.1727540

E-ISSN: 2458-9179
ADIYAMAN ÜNİVERSİTESİ SAĞLIK BİLİMLERİ DERGİSİ
JOURNAL OF HEALTH SCIENCES OF ADIYAMAN UNIVERSITY

Research Article/Özgün Araştırma

Chemical characterization and antiproliferative activity of bee venoms from Türkiye against NSCLC

Türkiye kökenli arı zehirlerinin kimyasal karakterizasyonu ve NSCLC'ye karşı antiproliferatif etkisi

Fatma ŞENGÜL BAĞ[1], Hüsamettin VATANSEV[2]

[1]Adıyaman University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Biochemistry Division, 02040, Adıyaman-Turkey
[2]Selçuk University, Faculty of Medicine, Department of Basic Medical Sciences, Biochemistry Division, 42130, Konya-Turkey

Atıf gösterme/Cite this article as: Şengül Bağ F, Vatansev H. Chemical characterization and antiproliferative activity of bee venoms from Türkiye against NSCLC. *ADYÜ Sağlık Bilimleri Derg*. 2025;11(3):205-214. doi:10.30569.adiyamansaglik.1727540

Abstract

Aim: This study compared the chemical composition and cytotoxic effect of bee venom samples from Adıyaman (A1), Bursa (B1) and a purified sample (H1) against A549 non-small cell lung cancer (NSCLC) cells.

Materials and Methods: Samples were filtered, lyophilized, and, for H1, further purified. The levels of melittin, PLA_2, and apamin were quantified by high-performance liquid chromatography (HPLC). Cytotoxicity was assessed in the A549 and HEK-293 cells using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay after 24 h of treatment, with cisplatin as a positive control. IC_{50} values were calculated to compare the potency of each sample and assess selectivity toward cancer cells.

Results: H1 had the highest melittin content (425.6 mg/L) and strongest cytotoxicity in A549 cells (IC_{50}: 1.964 ± 0.013 µg/mL), outperforming A1, B1 and cisplatin (p<0.0001).

Conclusion: H1, which is rich in melittin, showed strong antiproliferative activity on NSCLC cells with relatively low toxicity to normal cells, indicating its potential as a natural anticancer agent.

Keywords Bee venom; Melittin; A549 cells; Cytotoxicity; HPLC analysis.

Öz

Amaç: Bu çalışmada, Adıyaman (A1) ve Bursa (B1) illerinden elde edilen arı zehiri örnekleri ile saflaştırılmış bir örnek (H1)'in kimyasal bileşimi ve sitotoksik etkileri, A549 küçük hücre dışı akciğer kanseri (NSCLC) hücreleri üzerinde karşılaştırılmıştır.

Gereç ve Yöntem: Örnekler filtrelenmiş, liyofilize edilmiş ve H1 örneği için ek saflaştırma işlemi uygulanmıştır. Melittin, PLA_2 ve apamin düzeyleri yüksek performanslı sıvı kromatografi (HPLC) ile nicel olarak belirlenmiştir. Sitotoksisite, A549 ve HEK-293 hücrelerinde, 24 saatlik uygulama sonrasında, pozitif kontrol olarak sisplatin kullanılarak MTT [3-(4,5-dimetiltiazol-2-il)-2,5-difenil tetrazolyum bromür] testi ile değerlendirilmiştir. IC_{50} değerleri, her bir örneğin etkinliğini karşılaştırmak ve kanser hücrelerine yönelik seçiciliğini değerlendirmek amacıyla hesaplanmıştır.

Bulgular: H1 örneği en yüksek melittin içeriğine (425,6 mg/L) ve A549 hücrelerinde en güçlü sitotoksisiteye (IC_{50}: 1,964 ± 0,013 µg/mL) sahip olup A1, B1 ve sisplatinden anlamlı düzeyde daha etkilidir (p<0,0001).

Sonuç: Melittin açısından zengin olan H1 örneği, NSCLC hücrelerinde güçlü antiproliferatif aktivite göstermiş ve normal hücrelere karşı göreceli olarak düşük toksisite sergileyerek doğal bir antikanser ajan olma potansiyelini ortaya koymuştur.

Anahtar Kelimeler: Arı zehri; Melittin; A549 hücreleri; Sitotoksisite; HPLC analizi.

Yazışma Adresi/Address for Correspondence: Fatma ŞENGÜL BAĞ, Adıyaman University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Biochemistry Division, 02040, Adıyaman-Turkey, E-mail: fsengul@adiyaman.edu.tr

Geliş Tarihi/Received:25.06.2025 **Kabul Tarihi/Accepted:**04.09.2025 **Yayım Tarihi/Published online:**31.12.2025

Bu eser, Creative Commons Atıf-GayriTicari-AynıLisanslaPaylaş 4.0 Uluslararası Lisansı ile lisanslanmıştır
Telif Hakkı © 2025 Adıyaman Üniversitesi Sağlık Bilimleri Dergisi

Bu makale araştırma ve yayın etiğine uygun hazırlanmıştır.
intihal incelemesinden geçirilmiştir.

Introduction

Lung cancer remains the leading cause of cancer-related deaths worldwide, posing significant public health concern in terms of both incidence and mortality. According to the GLOBOCAN 2022 data published by the International Agency for Research on Cancer (IARC), a specialized agency of the World Health Organization (WHO), lung cancer accounted for 12.4% of all cancers, with approximately 2.5 million new cases, making it the most frequently diagnosed cancer type. Additionally, it was responsible for 1.8 million deaths, representing 18.7% of all cancer-related fatalities.[1] Histopathological, lung cancer is classified into two major groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC comprising approximately 85% of cases. NSCLC is characterized by a high metastatic potential and resistance to therapy, making it a significant clinical challenge.[2] Despite the use of conventional treatment modalities such as chemotherapy, radiotherapy, surgery, and immunotherapy in the management of NSCLC, these approaches often lack selectivity for cancer cells and may cause considerable damage to healthy tissues, leading to severe side effects. Although the bee venom samples tested in this study also showed cytotoxicity towards normal cells, their toxicity was relatively low compared to cisplatin, suggesting a potential advantage in the therapeutic index. Moreover, the emergence of drug resistance, limited therapeutic success, and increased treatment costs further underscore the urgent need for more targeted and effective treatment alternatives.[3] Bioactive compounds derived from natural sources (particularly natural toxins with low toxicity and selective anticancer potential) are gaining increasing attention as complementary or supportive biotherapeutic agents alongside existing treatment strategies.[4,5]

Bee venom is a complex mixture of biologically active molecules, primarily composed of peptides such as melittin, apamin, adolapin, and mast cell degranulating peptide, along with enzymes including phospholipase A_2 (PLA_2) and hyaluronidase. These components exhibit a wide range of pharmacological activities, including anti-inflammatory, antimicrobial, antinociceptive, immunomodulatory, and cytotoxic effects.[6] Preclinical studies have demonstrated that bee venom can exert selective cytotoxic effects against various cancer cell lines. Research focusing on lung cancer cell lines has also revealed promising results, indicating that bee venom may inhibit tumor growth and induce apoptosis.[7-10] However, the composition of bee venom can vary significantly depending on factors such as bee species, geographic origin, and even seasonal changes.[11,12] These variations may lead to differences in the biological activities of bee venoms derived from different sources. Therefore, comparative evaluation of the chemical profiles and cytotoxic potentials of distinct bee venom types is essential for identifying their therapeutic value.

The main objective of this study was to evaluate under in vitro conditions the cytotoxic effects of bee venom samples on the human lung cancer cell line A549 and to analyze their chemical compositions to show how differences in chemical content are associated with variations in cytotoxicity.

Materials and Methods

Chemicals

Bee venom samples were obtained from the Adıyaman Beekeepers Association (Adıyaman, Türkiye) and Dikbiyik Beekeeping Farm (Bursa, Türkiye). DMEM and DMEM/Ham's F-12 media used for cell culture experiments were purchased from Gibco (Life Technologies, Carlsbad, USA). Fetal bovine serum (FBS, Cat. No: P30-193306) was obtained from PAN Biotech, and L-glutamine (200 mM, Cat. No: 25030-081) was acquired by Thermo Fisher Scientific. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], DMSO (dimethyl sulfoxide), trypan blue, 0.25% trypsin–EDTA solution, and penicillin–streptomycin antibiotic mixture was all procured from Sigma (St. Louis, USA).

Preparation of bee venom samples

Bee venom samples from Adıyaman (A1) and Bursa (B1) were first dissolved in pyrogen-free water and filtered through coarse-grade filter paper (Whatman® qualitative filter paper, Grade 1; pore size ~11 µm) using a funnel to remove particulates such as pollen, fecal residues, dust, nectar, or honey. The filtrates were then passed through sterile membrane filters (pore size 0.22 µm) to eliminate pyrogenic contaminants and lyophilized. A portion of each sample was submitted to the Duzce University Scientific and Technological Research Application and Research Centre (DUBIT) for compositional analysis, while the remaining aliquots were stored at −20 °C until further experimentation. The B1 sample was not subjected to additional purification and was used in its lyophilized form after coarse and membrane filtration steps.

Purification of bee venom

The A1 sample was subjected to an additional purification step to isolate melittin, apamin and phospholipase A_2 (PLA_2). In brief, approximately 50 mL of filtrate from the coarse filter paper (Whatman® qualitative filter paper, Grade 1; pore size ~11 µm) filtration and 50 mL of hexane (≥99% purity) were mixed and transferred to a separatory funnel. The mixture was allowed to stand for approximately 1 hour to enable phase separation. The liquid phase was collected, and the hexane portion was evaporated at 37 °C for 40 minutes. The remaining solution was passed through a sterile 0.22 µm syringe filter to remove pyrogenic bacteria and then lyophilized. The pH of the bee venom solution was measured before lyophilization and was found to be 4.5–5.5, which is consistent with literature values.[13] The purified sample (H1) was weighed into 1 mg sterile vials with colored caps, sealed and stored at −20 °C until analysis.

HPLC-based compositional analysis

Quantitative analysis of bioactive components in bee venom samples was performed using high-performance liquid chromatography (HPLC) as described by Kekeçoğlu et al. with minor modifications.[12] A Shimadzu system equipped with an LC-20AT pump, SIL-20HT autosampler, SPD-M20A detector, and CTO-10AS column oven was used. Separation was achieved with a C18 reverse-phase column (4.6 × 250 mm, 5 µm). A gradient method was applied using mobile phase A (0.1% trifluoroacetic acid in ultrapure water) and mobile phase B (0.1% TFA in an 80:20 mixture of acetonitrile and ultrapure water). Mobile phase B increased from 5% to 80% over 30 minutes. The column temperature was maintained at 25 °C, with a flow rate of 1 mL/min and injection volume of 20 µL. UV/VIS detection was performed at 220 nm. All analyses were conducted in triplicate.

Commercially available purified standards of melittin (100 mg/L), apamin (100 mg/L) and PLA_2 (250 mg/L) (Sigma-Aldrich, USA) were used to generate calibration curves (R^2>0.99), and peak identification was confirmed by matching retention times and UV spectra with those of the standards. In this study, the compositional analysis was limited to these three primary bioactive peptides. Other components of the bee venom, such as free amino acids, small organic acids, additional bioactive peptides and other venom proteins, were not quantified.

The concentrations of honeybee venom components were derived using equations generated from their respective standard calibration curves. Concentrations of melittin, PLA_2, and apamin were calculated from calibration curves prepared with purified standards and expressed in milligrams per liter (mg/L) of sample solution.

Cell culture conditions

The study utilized A549 human lung adenocarcinoma and HEK-293 healthy human embryonic kidney cell lines. A549 cells were cultured in DMEM/Ham's F-12, while HEK-293 cells were maintained in DMEM medium. Both media were supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin–streptomycin. Cells were incubated under standard conditions (37 °C, 5% CO_2, humidified atmosphere). All procedures were performed under sterile conditions using a laminar flow cabinet.

A549 cells were selected as an established model for primary lung cancer to investigate

the antitumor potential of bee venom samples. HEK-293 cells were used as a non-cancerous control to assess general cytotoxicity, as no normal lung-derived cell line was available in our laboratory at the time of the experiments. Instead, HEK-293 cells were selected because they are routinely maintained at our institution, have robust growth characteristics, and are frequently used in cytotoxicity screening studies.

Cell viability (MTT) assay procedure

A549 and HEK-293 cells were seeded into 96-well plates at a density of 5×10^3 cells per well in 100 µL of the appropriate culture medium. After 24 hours of incubation to allow for adherence, the media were replaced with treatment solutions. A1 and B1 venom samples were applied to A549 cells at concentrations of 0.5, 2, 8, and 16 µg/mL, while H1 was tested at 0.31, 0.625, 1.25, and 2.5 µg/mL. Cisplatin, used as positive control, was tested at concentrations of 3.125, 6.25, 12.5, and 25 µg/mL. The concentration ranges for the bee venom samples were selected based on literature data[9,10,14] and preliminary experiments conducted in our laboratory to determine threshold values for cytotoxicity. Each condition was tested in triplicate. Following 24 hours of exposure, 20 µL of freshly prepared MTT solution (5 mg/mL) was added to each well and incubated at 37 °C for 3–4 hours. The medium was then removed, and 150 µL of DMSO was added to dissolve the formazan crystals. Plates were incubated in the dark on a shaker for 15–20 minutes, and absorbance was measured at 570 nm using a CLARIOstar microplate reader (BMG Labtech, Germany). Cell viability percentages were calculated by comparing absorbance values to those of the untreated control group.

Toxicity assessment on normal cells

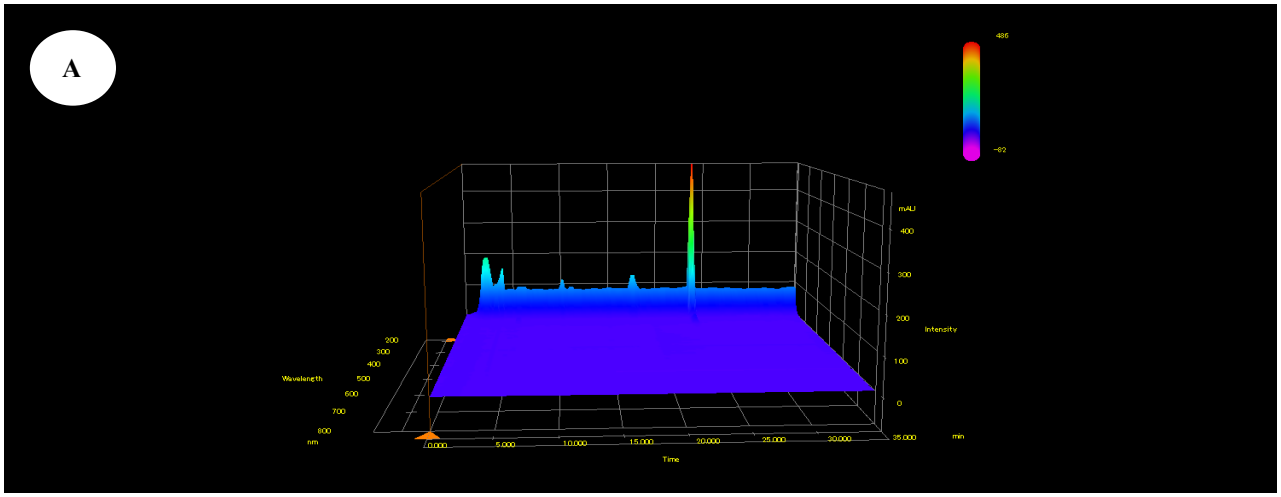
IC_{50} values were determined for each venom sample and cisplatin in A549 cells. These concentrations were subsequently applied to HEK-293 cells under identical assay conditions to assess cytotoxicity in normal cells.

Statistical Analysis

The results of the cell viability assays were presented as mean ± standard deviation (SD) from three independent experiments. Intergroup comparisons were conducted using one-way ANOVA, followed by Tukey's post-hoc test for pairwise comparisons when overall significance was observed. Statistical analysis was performed using GraphPad Prism version 10.5 (GraphPad Software Inc., San Diego, CA, USA), and a p-value below 0.05 was considered statistically significant.

Results

The HPLC chromatograms of the bee venom samples A1, B1, and H1 are presented in Figure 1. According to the analysis results, melittin, PLA_2, and apamin concentrations in the A1 sample (Fig. 1A) were 383.98 mg/L, 59.46 mg/L, and 10.78 mg/L, respectively. In the B1 sample (Fig. 1B), melittin was detected at 391.02 mg/L, PLA_2 at 64.74 mg/L, and apamin at 12.82 mg/L. The purified H1 sample (Fig. 1C) showed the highest melittin content at 425.60 mg/L, followed by PLA_2 at 56.72 mg/L and apamin at 10.94 mg/L.



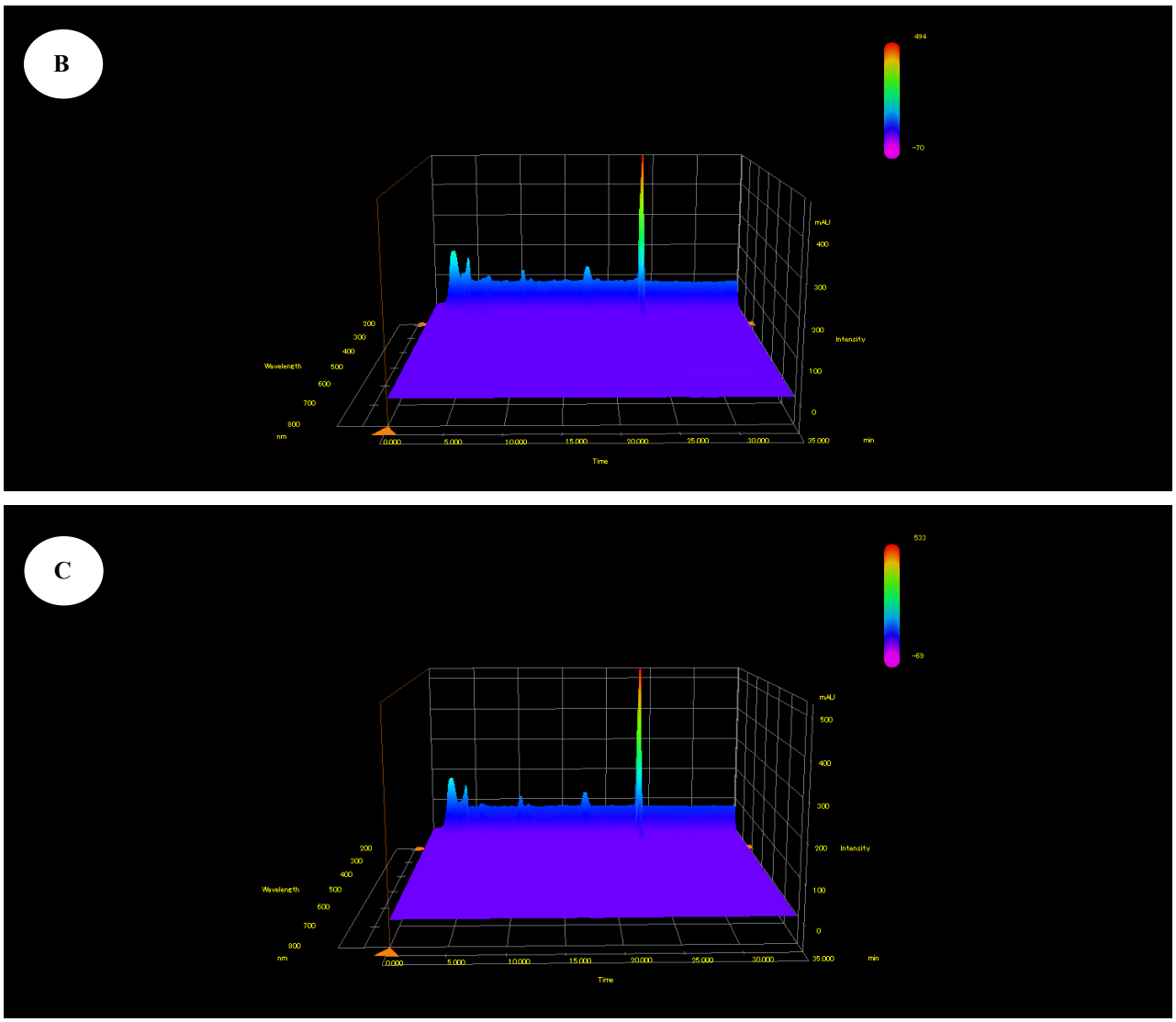


Figure 1. Three-dimensional HPLC chromatograms showing retention time (min), wavelength (nm), and absorbance intensity (mAU) of bee venom samples: (A) A1 – bee venom from Adıyaman, (B) B1 – bee venom from Bursa, and (C) H1 – purified fraction from A1 sample.

As shown in Fig. 2, apamin, PLA_2, and melittin peaks were clearly identified in all venom samples, with melittin exhibiting the most prominent peaks, indicating its role as the major component.

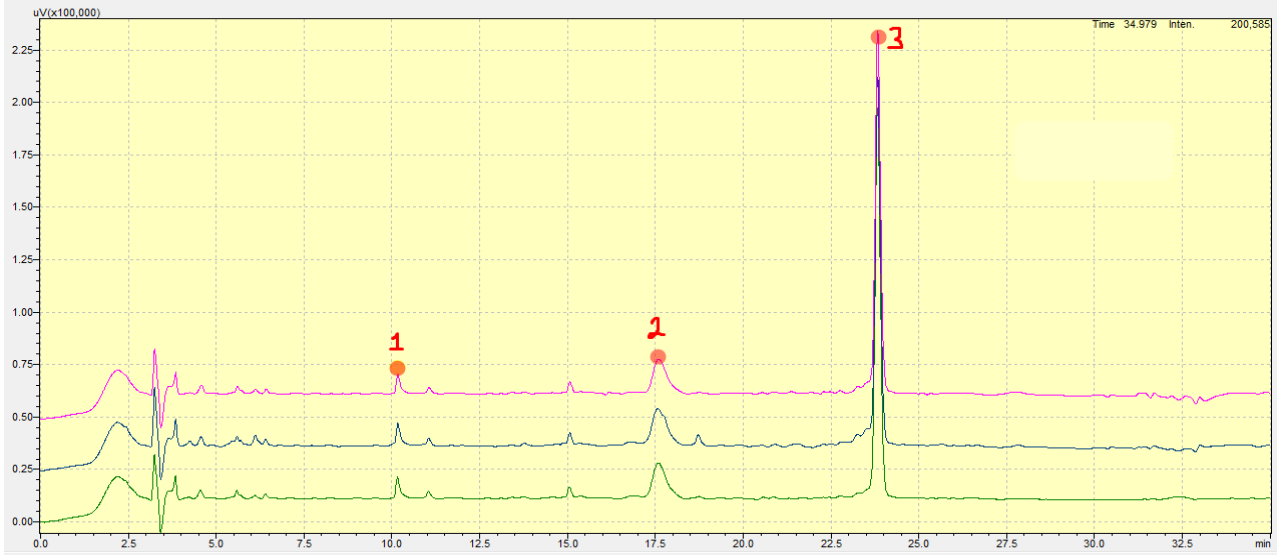


Figure 2. HPLC chromatograms of bee venom samples: (1) Apamin, (2) PLA2, (3) Melittin.

The cytotoxic effects of different bee venom samples (H1, A1, B1) on A549 lung adenocarcinoma cells were assessed via MTT assay. As illustrated in Fig. 3A, the dose-response curve for the H1 sample revealed a potent cytotoxic effect, with an IC_{50} value of 1.964 ± 0.013 µg/mL (Fig. 3B), indicating the strongest antiproliferative activity among the tested samples.

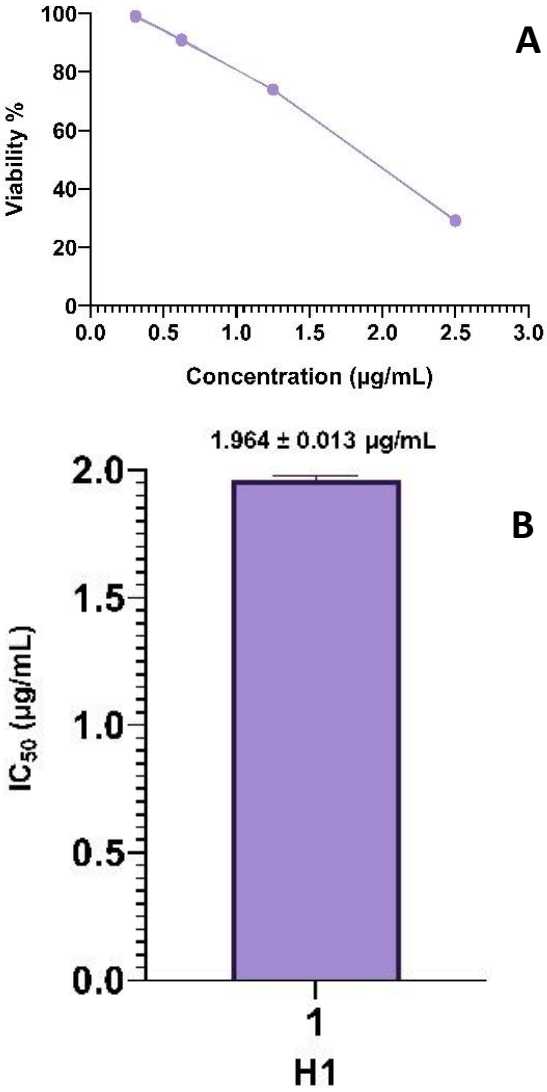


Figure 3. (A) Cell viability percentages of A549 cells treated with various concentrations of the H1 sample. (B) IC_{50} value of the H1 sample.

Dose-response curves for the A1 and B1 samples are shown in Fig. 4A. The IC_{50} values were calculated as 14.6 ± 0.13 µg/mL for A1 and 15.407 ± 0.155 µg/mL for B1 (Fig. 4B). These findings indicate that the H1 sample exerts a stronger cytotoxic effect even at lower concentrations compared to A1 and B1.

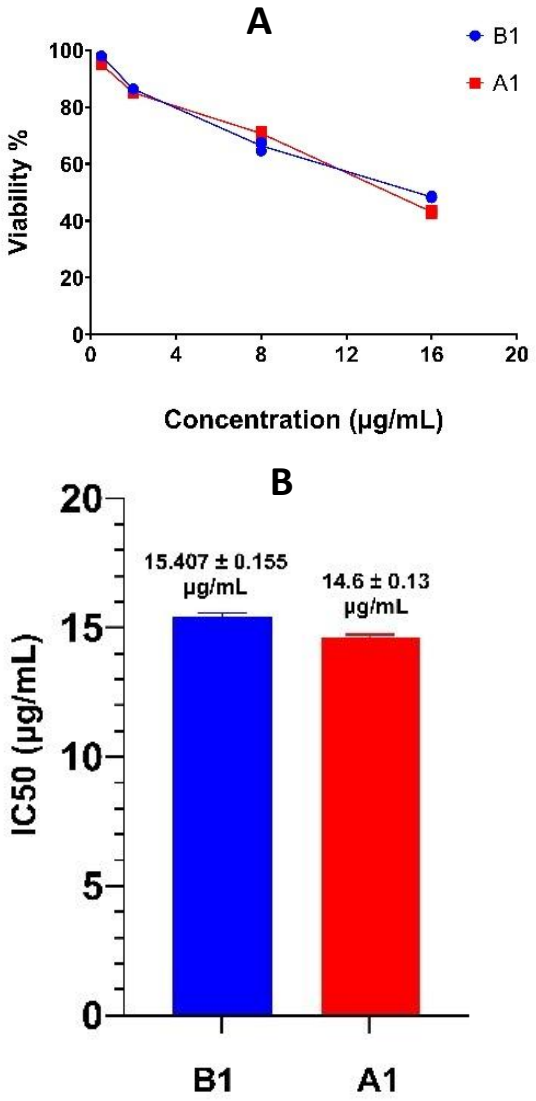
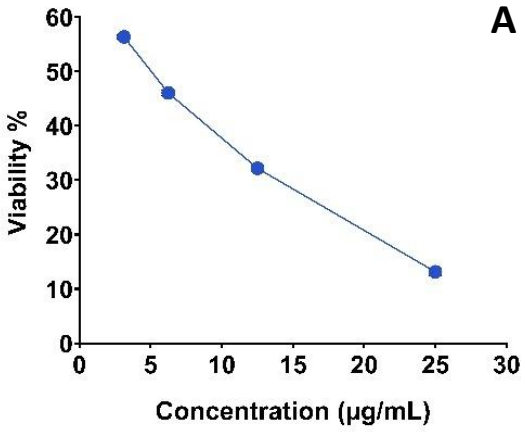


Figure 4. (A) Cell viability percentages of A549 cells treated with different concentrations of A1 and B1 samples. (B) IC_{50} values of A1 and B1 samples.

Cisplatin, used as a positive control, exhibited an IC_{50} value of 4.978 ± 0.136 µg/mL. The dose-response curve and IC_{50} value of cisplatin are shown in Fig. 5A and 5B, respectively.



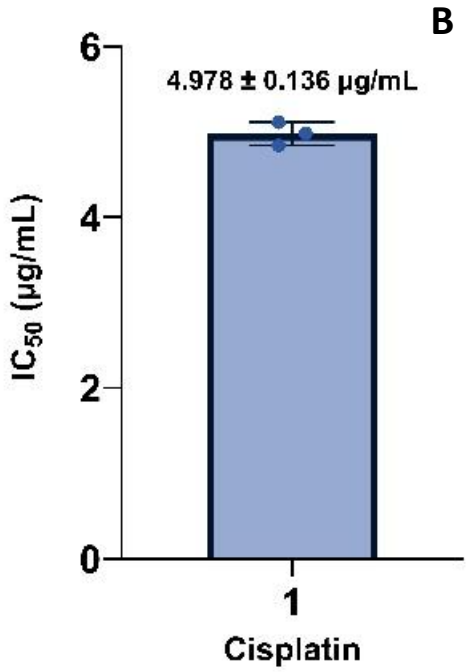


Figure 5. (A) Cell viability percentages of A549 cells treated with varying concentrations of cisplatin. (B) IC_{50} value of cisplatin.

Among all tested samples, the H1 sample showed the lowest IC_{50} value and significantly greater cytotoxicity compared to all other groups (p<0.0001), followed by cisplatin, B1, and A1. A concentration-dependent decline in cell viability was observed for all samples (Fig. 6). When IC_{50} doses of each sample were applied to healthy HEK-293 cells, cell viability was measured as 71.067 ± 1.137% for H1, 84.733 ± 0.611% for A1, 81.7 ± 1.513% for B1, and 42.3 ± 1.136% for cisplatin.

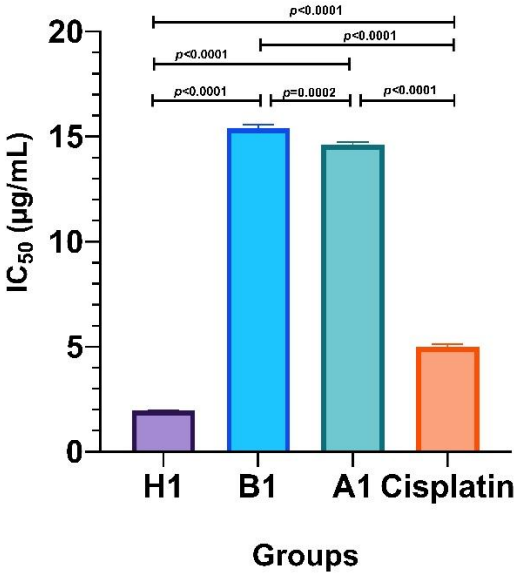


Figure 6. Comparative IC_{50} values (µg/mL) of H1, B1, A1 samples, and cisplatin on A549 cells.

Discussion

This study provides a novel comparative analysis of bee venom samples from different geographical regions of Türkiye, focusing on their chemical composition and cytotoxic potential against human lung adenocarcinoma cells. While the therapeutic and anticancer properties of bee venom have been extensively documented, there are few studies that directly compare the bioactivity of venom from different regions of the same country under standardized experimental conditions. Such comparisons are important because the composition of bee venom is influenced by local flora, climate and subspecies of bees, all of which can alter the relative abundance of biologically active peptides such as melittin, apamin and PLA_2. Understanding these regional differences is critical not only for identifying highly potent venom sources for pharmaceutical applications, but also for establishing standardization protocols to ensure reproducible therapeutic effects.

In the present study, the cytotoxic effects of bee venom samples (A1, B1, and H1) obtained from different geographical regions (Adıyaman and Bursa, Türkiye) were evaluated on the human lung adenocarcinoma cell line A549, and the relationship between these effects and the chemical composition of the venom was investigated. The presence and relative proportions of major components such as melittin, apamin, and PLA_2 were quantitatively analyzed, and their effects on cell viability were assessed using the MTT assay. Additionally, IC_{50} values determined in the A549 cells were applied to healthy human embryonic kidney (HEK-293) cells to assess comparative toxicity profiles.

HPLC analysis revealed that the bee venom samples, particularly the purified H1 sample, contained significantly higher concentrations of melittin compared to values reported in previous studies.[12,15,16] This observation suggests that the biological composition of bee venom may vary substantially depending on regional flora, bee species, and environmental conditions. Melittin exerts its cytotoxic effect by binding to phospholipid membranes, disrupting membrane integrity, and altering intracellular ion homeostasis, ultimately

inducing cell death. Moreover, melittin is known to exhibit synergistic anticancer effects when acting in combination with other venom components such as PLA_2 and apamin.[4,16]

According to the MTT assay results, the IC_{50} value of the H1 sample was calculated as 1.964 ± 0.013 µg/mL, which was significantly lower than that of A1 (14.6 ± 0.13 µg/mL), B1 (15.407 ± 0.155 µg/mL), and the positive control cisplatin (4.978 ± 0.136 µg/mL). These findings underscore the potent antiproliferative effect of the H1 sample on A549 cells. With a melittin content of 425.6 µg/mL, the H1 sample displayed the highest concentration among all tested samples, further supporting the central role of melittin in exerting cytotoxic effects. The relatively higher IC_{50} values of A1 and B1 may be attributed to their lower melittin and PLA_2 concentrations. Such compositional differences may result from environmental variables such as geographical origin (Adıyaman vs. Bursa), seasonal variation, and differences in local flora and bee species.

Cisplatin was used as a positive control in A549 cells and exhibited an IC_{50} value of 4.978 ± 0.136 µg/mL, which corresponds to a concentration lower than its clinical dose (9.033 µg/mL or 30 µM).[17] In previous reports, Dai et al. calculated the IC_{50} of cisplatin in A549 cells as 4.97 ± 0.32 µg/mL[18], while a pharmacogenomic database reported a value of 6.097 µg/mL (20 µM). The cisplatin value observed in our study was consistent with both the literature and database findings. Notably, the H1 sample exhibited an even lower IC_{50} value, suggesting that specific bee venom fractions may have superior efficacy against cancer cells. When the same IC_{50} concentrations were applied to HEK-293 cells, cell viability was measured as 71.067 ± 1.137% for H1, 84.733 ± 0.611% for A1, 81.7 ± 1.513% for B1, and 42.3 ± 1.136% for cisplatin, indicating that bee venom demonstrates a relatively selective cytotoxic effect with reduced toxicity toward normal cells.

The antiproliferative effects of bee venom on lung cancer cells have been extensively supported by numerous studies in literature.[7,8,10,14,19] Research on NSCLC cell lines has shown that these effects are not limited to direct cytotoxicity but also involve the modulation of intracellular signaling pathways.[9,10] In a study conducted by Choi et al., bee venom (1–5 µg/mL) was shown to inhibit cell proliferation and induce apoptosis in A549 and NCI-H460 cell lines in a dose-dependent manner.[10] This effect was mediated by increased expression of death receptors DR3 and DR6 and was associated with the inactivation of the NF-κB pathway. Furthermore, silencing DR3 expression via siRNA significantly reversed the growth-inhibitory effect of bee venom.[10] Melittin, a major component of bee venom, has been reported to suppress tumor development by inhibiting the TGF-β/ERK signaling pathway, thereby reducing cell proliferation and invasion in NSCLC cells. In a study by Yu et al., melittin was found to inhibit migration in A549 and H358 cell lines, decrease TGF-β expression and phospho-ERK levels, and significantly reduce tumor volume in in vivo models.[9] Similarly, Zhang and Chen demonstrated that melittin suppressed EGF-induced cell migration, reduced VEGF and HIF-1α expression, and exerted anti-angiogenic effects, leading to a 27–61% reduction in tumor volume in vivo.[8] In NCI-H1299 lung cancer cells, bee venom increased caspase-3 activity, upregulated Bax expression, and downregulated Bcl-2 levels. Additionally, it was shown to inhibit COX-2 mRNA expression and PGE2 production.[19] In our previous study using the A1 sample, bee venom elevated Bax levels while decreasing Bcl-2 expression.[7] When combined with mesenchymal stem cells, bee venom exhibited a synergistic effect, contributing further to its anticancer potential.[7] Moreover, a study by Badavi et al. revealed that a PEGylated liposomal bee venom formulation showed a lower IC_{50} value and more effective suppression of cell proliferation in A549 cells compared to free bee venom.[14]

The biological activity of melittin extends beyond direct cytotoxicity. It has also been associated with anti-inflammatory effects, including suppression of cytokine expression, NF-κB inactivation, and modulation of cellular signaling pathways.[20] Sadek et al. emphasized

that the composition of bee venom varies with geographical, seasonal, and biological factors, highlighting the importance of standardization for pharmaceutical applications.[11] Additionally, our content analysis suggests that bee venom samples from Türkiye—especially those obtained from Adıyaman and Bursa—may have higher melittin concentrations than those reported in studies from countries such as Morocco and Portugal.[15,21]

Limitations

This study has several limitations. First, only one cancer cell line (A549) and one normal cell line (HEK-293) were used, without including other NSCLC subtypes or in vivo models. Second, cell death mechanisms were assessed solely using the MTT assay, without performing molecular analyses related to apoptosis or other forms of cell death. Third, although comparisons were made with cisplatin, no evaluation was conducted against other chemotherapeutic agents. While the H1 sample was enriched, individual compounds such as melittin and PLA_2 were not isolated and tested separately, limiting mechanistic interpretations. In addition, the lack of mechanistic studies, which are standard in cytotoxicity studies, further limits the interpretability of the results. This study is also limited to the quantification of the three main bee venom components (melittin, PLA_2 and apamin). Other potentially relevant components, including free amino acids, small organic acids and additional bioactive peptides, were not analyzed. Future studies encompassing a broader range of compounds may provide a more comprehensive chemical characterization and mechanistic understanding. Lastly, given that the chemical composition of bee venom varies with geographical and environmental factors, standardization remains a challenge for clinical applicability.

Conclusion

The results of this study show that bee venom samples with high melittin content exert a strong cytotoxic effect on NSCLC cells without causing significant toxicity to healthy cells. This study demonstrates the importance of correlating the chemical composition of bee venom with its biological effects and suggests that, pending further mechanistic and in vivo investigations, this natural product has the potential to support or complement cancer treatment rather than being directly classified as a targeted cancer therapy. Future research should focus on elucidating the molecular mechanisms underlying these effects, validating efficacy in in vivo NSCLC models and evaluating the therapeutic index under physiological conditions. Given the variability in bee venom composition due to environmental and geographic factors, the development of standardized extraction and purification protocols will be critical to ensure reproducible and clinically actionable results. The promotion of bee venom production in Türkiye could support the domestic biotechnology sector and improve competitiveness in the global pharmaceutical market. At a time of growing interest in natural products, research uncovering the therapeutic potential of bioactive compounds such as melittin underscores the strategic importance of bee venom. With more detailed insights into its mechanisms of action, it may be possible to develop novel, low-toxicity and targeted therapies that position bee venom as a valuable resource in both medical and commercial contexts.

Ethics Committee Approval

In the current work, there was no animal or human experiments conducted.

Author Contributions

All authors contributed equally to the design of the study, the execution of the experiments, the analysis of the data, and the writing of the manuscript. The results were jointly discussed, and the final version of the manuscript was approved by all authors.

Acknowledgements

This study was financially supported by the Scientific Research Projects Coordination Office of Selçuk University under project number 21112006. The authors gratefully acknowledge the support provided by the institution.

Conflicts of Interest

The authors declare that there is no conflict of interest for this article.

Financial Disclosure

This study was financially supported by the Scientific Research Projects Coordination Office of Selçuk University under the project number 21112006.

Statements

Part of the data on the chemical composition of the A1 bee venom sample has already been reported in brief.[7] However, the comparative analysis, the extended cytotoxicity tests and the results with the B1 and H1 samples presented in this study are original and have not yet been published or submitted elsewhere.

Peer-review

Externally peer-reviewed.

References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*. 2024;74(3):229-263.
2. Padinharayil H, Varghese J, John MC, et al. Non-small cell lung carcinoma (NSCLC): Implications on molecular pathology and advances in early diagnostics and therapeutics. *Genes & diseases*. 2023;10(3):960-989.
3. Li Y, Yan B, He S. Advances and challenges in the treatment of lung cancer. *Biomedicine & Pharmacotherapy*. 2023;169:115891.
4. Oršolić N. Bee venom in cancer therapy. *Cancer and Metastasis Reviews*. 2012;31(1):173-194.
5. Zhang Y. Why do we study animal toxins? *Dongwuxue Yanjiu*. 2015;36(4):183-222.
6. Şengül F, Vatansev H. Overview of apitherapy products: anti-cancer effects of bee venom used in apitherapy. *International Journal of Traditional and Complementary Medicine Research*. 2021;2(1):36-48.
7. Sengul F, Vatansev H, Ozturk B. Investigation the effects of bee venom and H-dental-derived mesenchymal stem cells on non-small cell lung cancer cells (A549). *Molecular Biology Reports*. 2023;51(1):2.
8. Zhang SF, Chen Z. Melittin exerts an antitumor effect on non-small cell lung cancer cells. *Molecular medicine reports*. 2017;16(3):3581-3586.
9. Yu R, Wang M, Wang M, Han L. Melittin suppresses growth and induces apoptosis of non-small-cell lung cancer cells via down-regulation of TGF-β-mediated ERK signal pathway. *Brazilian journal of medical and biological research*. 2020;54(2):e9017.
10. Choi KE, Hwang CJ, Gu SM, et al. Cancer cell growth inhibitory effect of bee venom via increase of death receptor 3 expression and inactivation of NF-kappa B in NSCLC cells. *Toxins (Basel)*. 2014;6(8):2210-28.
11. Sadek KM, Shib NA, Taher ES, et al. Harnessing the power of bee venom for therapeutic and regenerative medical applications: an updated review. *Frontiers in pharmacology*. 2024;15:1412245.
12. Kekeçoğlu M, Çaprazlı, T., Samancı, A. E. T., et al. Factors affecting quality of honey bee venom. *Journal of Apicultural Science*. 2022;66(1):5-14.
13. Khalil A, Elesawy BH, Ali TM, et al. Bee Venom: From Venom to Drug. *Molecules*. 2021;26(16):4941.
14. Badivi S, Kazemi S, Eskandarisani M, et al. Targeted delivery of bee venom to A549 lung cancer cells by PEGylate liposomal formulation: an apoptotic investigation. *Scientific reports*. 2024;14(1):17302.
15. Sobral F, Sampaio A, Falcão S, et al. Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*. 2016;94:172-7.
16. Wehbe R, Frangieh J, Rima M, El Obeid D, Sabatier JM, Fajloun Z. Bee Venom: Overview of Main Compounds and Bioactivities for Therapeutic Interests. *Molecules*. 2019;24(16):2997.
17. Huang Z, Tong Y, Wang J, Huang Y. NMR studies of the relationship between the changes of membrane lipids and the cisplatin-resistance of A549/DDP cells. *Cancer Cell International*. 2003;3(1):5.
18. Dai CH, Li J, Chen P, Jiang HG, Wu M, Chen YC. RNA interferences targeting the Fanconi anemia/BRCA pathway upstream genes reverse cisplatin resistance in drug-resistant lung cancer cells. *Journal of biomedical science*. 2015;22(1):77.
19. Jang MH, Shin MC, Lim S, et al. Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. *Journal of pharmacological sciences*. 2003;91(2):95-104.
20. Lee G, Bae H. Anti-Inflammatory Applications of Melittin, a Major Component of Bee Venom: Detailed Mechanism of Action and Adverse Effects. *Molecules*. 2016;21(5):616.
21. El Mehdi I, Falcão SI, Harandou M, et al. Chemical, Cytotoxic, and Anti-Inflammatory Assessment of Honey Bee Venom from Apis mellifera intermissa. *Antibiotics (Basel)*. 2021;10(12):1514.