RESEARCH ARTICLE
Bull Biotechnol (2024) 5(2):44-50
https://doi.org/10.51539/biotech.1563918 Journal homepage: www.dergipark.org.tr/biotech

Bulletin of Biotechnology

Differential cytotoxicity of methanol and water extracts from *Bacopa monnieri* (L.) Wettst and *Ceratophyllum demersum* L. on HepG2 and THLE2 cells

Mustafa Ari[1], Bugrahan Emsen[1*], Muhammet Dogan[2]

[*1]*Department of Biology, Kamil Özdağ Faculty of Science, Karamanoğlu Mehmetbey University, Karaman, Türkiye*
[2]*Department of Nutrition and Dietetics, Faculty of Health Sciences, Karamanoğlu Mehmetbey University, Karaman, Türkiye*

*Corresponding author : bugrahanemsen@gmail.com
Orcid No: https://orcid.org/0000-0002-9636-2596

Received : 09/10/2024
Accepted : 08/12/2024

To Cite: Ari M, Emsen B, Dogan M (2024) Differential cytotoxicity of methanol and water extracts from *Bacopa monnieri* (L.) Wettst and *Ceratophyllum demersum* L. on HepG2 and THLE2 cells. Bull Biotechnol 5(2):44-50 https://doi.org/10.51539/biotech.1563918

Abstract: This study evaluated the cytotoxic effects of methanol and water extracts from *Bacopa monnieri* (L.) Wettst and *Ceratophyllum demersum* L. on HepG2 (liver cancer) and THLE2 (normal liver) cell lines using XTT assay. The extracts were tested at concentration range of 12.5-400 µg/mL. In HepG2 cells, the water extract of *B. monnieri* at 400 µg/mL exhibited the highest cytotoxicity, reducing cell viability to 11.08%, which was statistically significant ($p < 0.05$) compared to other treatments. The methanol extract of *C. demersum* at 12.5 µg/mL had the least effect, maintaining 94.84% cell viability. For THLE2 cells, the water extract of *B. monnieri* (400 µg/mL) also showed the greatest reduction in cell viability (30.85%). The water extract of *C. demersum* at the same concentration resulted in similar viability (32.01%), with no significant statistical difference ($p > 0.05$). The lowest concentrations of methanol and water extracts of *C. demersum* showed minimal effects (97.55% and 97.74% viability, respectively). Median inhibitor concentration (IC_{50}) analysis revealed that *B. monnieri*'s water extract was most effective, with IC_{50} values of 68.45 µg/mL for HepG2 and 127.05 µg/mL for THLE2 cells. In contrast, *C. demersum*'s methanol extract had the highest IC_{50} values (173.35 µg/mL and 228.46 µg/mL, respectively), indicating lower cytotoxicity. Heatmap and cluster analyses highlighted the selective cytotoxicity of *C. demersum* on cancer cells with minimal effects on normal cells, showing its potential for targeted cancer therapy.

Keywords: anticancer activity; cell line comparison; extract potency; selective toxicity

1 Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer that begins in hepatocytes, the main cells of the liver. This disease is often a major cause of liver failure (García-Pras et al. 2021). The early stages of HCC are usually asymptomatic, but as it progresses, symptoms such as abdominal pain, loss of appetite, weight loss, jaundice, and abdominal swelling may occur. Factors such as hepatitis B and C virus infections, alcohol consumption, obesity, and diabetes increase the risk of HCC (Ruiz-Margáin et al. 2021). Blood tests, imaging techniques, and biopsy are used for diagnosis. Treatment options include surgery, radiotherapy, chemotherapy, ablation, and liver transplantation. The prognosis of HCC depends on the stage of the cancer and the success of treatment (Granata et al. 2021). Early diagnosis and treatment have a significant impact on the course of the disease. The risk of HCC can be reduced through hepatitis B and C vaccinations, a healthy lifestyle, and regular health check-ups (Flores et al. 2022).

The use of herbal products in the treatment of serious diseases such as cancer is an important topic that has been researched both historically and in modern times. While plants have been used to treat various ailments for thousands of years, modern medicine relies on evidence-based methods for the treatment of cancer (Dutta et al. 2019; Sharma et al. 2024). In this context, some herbal products can help reduce side effects such as nausea, vomiting, and fatigue caused by conventional treatments like chemotherapy or radiotherapy (Yazbeck et al. 2022; Bai et al. 2024). Certain plants may support the body's fight against cancer by strengthening the immune system. Additionally, some plants contain compounds that exhibit cytotoxic effects on cancer cells, inhibiting their growth or inducing cell death. This is one reason why plants have been used in traditional medicine for centuries to treat various diseases (Garcia-Oliveira et al. 2021; Esmeeta et al. 2022).

Bacopa monnieri (L.) Wettst has been used for many years in the treatment of cognitive problems such as memory and learning difficulties (Brimson et al. 2021). However, recent

studies have shown that *B. monnieri* may also have potential in cancer treatment. *B. monnieri* is a powerful antioxidant. Antioxidants prevent cell damage by neutralizing free radicals in the body, thus reducing the risk of cancer (Ghosh et al. 2021; Fatima et al. 2022). Chronic inflammation is associated with the development of many types of cancer, and the anti-inflammatory properties of *B. monnieri* may play a beneficial role in this process. Some studies suggest that *B. monnieri* may trigger programmed cell death (apoptosis) in cancer cells, potentially slowing or stopping tumor growth (Mishra et al. 2024).

Ceratophyllum demersum L., commonly known as the marsh flower, is a fully aquatic plant species (Engloner et al. 2023). This plant plays an important role in underwater ecosystems, but in recent years, it has attracted the attention of scientists due to its potential anticancer compounds. Some studies have shown that *C. demersum* extracts inhibit growth and promote cell death in various cancer cell lines (Masłyk et al. 2024). In particular, some research suggests that this plant is effective against colon, liver, and breast cancer cells (Saxena et al. 2021).

In cases where it is difficult to reproduce plants in natural environments, such as *B. monnieri* and *C. demersum*, tissue culture is a highly advantageous method. Since plants produced via tissue culture are genetically identical, the amount and type of bioactive compounds they contain are more homogeneous (Zuzarte et al. 2024). This provides a significant advantage in medical research and product development. Tissue culture offers great potential for the use of plants like *B. monnieri* and *C. demersum* for medical purposes. Through this method, it is possible to obtain standardized, disease-free, and large quantities of plant material (Haque et al. 2022; Jain et al. 2023).

HCC was likely chosen for this study due to its status as the most common type of liver cancer and a major cause of liver failure. This type of cancer has high mortality rates, particularly in later stages when treatment options become limited and less effective (Schlachterman et al. 2015). Additionally, HCC is often linked to hepatitis B and C infections, making it a type of cancer with known viral associations, especially in regions where these infections are prevalent (Min et al. 2023; Stroffolini and Stroffolini, 2023). By researching HCC specifically, scientists may find new ways to improve outcomes using alternative treatments, like herbal products from *B. monnieri* and *C. demersum*, which are thought to support cancer treatment by reducing side effects and possibly slowing cancer cell growth. In the present study, we examined the cytotoxic effects of different extracts obtained from *B. monnieri* and *C. demersum*, which were produced using tissue culture techniques, on human hepatocellular carcinoma (HepG2) cells. In addition, we tested side effect levels of the extracts on non-tumoral human liver (THLE2) cells.

2 Materials and Method

2.1 Plant tissue culture

In this study, sterile *C. demersum* and *B.monnieri* were obtained from the Biology Department of Karamanoğlu Mehmetbey University as plant material. Shoot tip explants from sterile and stock plants were isolated under sterile conditions and placed into the nutrient medium. Murashige and Skoog (MS) mineral salts and vitamins (Murashige and Skoog, 1968) were used as the nutrient medium in all experiments. As a plant growth regulator, 1 mg/L Benzyl Amino Purine (BAP) and 30 g/L sucrose were added in all trials. Experiments with *C. demersum* were conducted in a liquid medium without agar (Emsen and Dogan, 2018), while those with *B. monnieri* were performed in a medium containing 7 g/L agar (Dogan and Emsen, 2018; Dogan and Ugur, 2024). The pH of the nutrient medium was adjusted to between 5.6 and 5.8 using 1 N NaOH or KOH, or 1 N HCl, and then sterilized by autoclaving at 121°C and 1.2 atmospheres of pressure for 20 minutes. The cultures were placed in a plant growth chamber or room and incubated under a 16-hour light/8-hour dark cycle at a temperature of 24±1°C.

2.2 Preparation of the extracts

The plant samples (10 g) of *B. monnieri* and *C. demersum* were dried, ground into powder, and then extracted with methanol and water solvents (250 mL) using a Soxhlet extractor. The extracts were filtered, the solvent was removed, and the resulting dry powders were lyophilized (Emsen and Dogan, 2018).

2.3 Cell culture

Human liver cells, both cancerous (HepG2) and non-cancerous (THLE2), were grown in a Dulbecco's Modified Eagle Medium (DMEM) under specific conditions (with 10% heat-inactivated fetal bovine serum (FBS), 1% l-glutamine and 1% penicillin–streptomycin). Once the cells reached a certain density, they were used for experiments to test the toxicity of the samples being studied (Emsen et al. 2021).

2.4 Cytotoxic activity

Cells were plated at a density of 1×10^4 cells per well in 96-well flat-bottom microtiter plates and incubated at 37°C for 24 hours. After this incubation period, the medium was replaced with fresh medium containing various concentrations of plant extracts. The final extract concentrations in the wells were 12.5, 25, 50, 100, 200, and 400 µg/mL, with 0.5% dimethyl sulfoxide (DMSO) serving as the negative control. Following a 48-hour incubation, XTT reagent and activator (from Biological Industries, Beit Haemek, Israel) were added to each well as per the manufacturer's protocol. The plates were then incubated for an additional 4 hours at 37°C. Absorbance readings were taken at 450 nm using a spectrophotometer, with a blank serving as the background control. Cell viability was determined as a percentage using the formula: Viability = (Absorbance of extract / Absorbance of control) × 100 (Kok et al. 2023).

2.5 Statistical analyses

The extract activities were evaluated using one-way ANOVA followed by Duncan's test for post-hoc comparison. Probit regression analysis was employed to determine the median inhibitory concentration (IC_{50}) values. To explore the similarities and differences in cytotoxic activities, heatmap

analysis and hierarchical cluster analysis were performed using Ward's minimum variance method. All statistical analyses were conducted using SPSS software (version 21.0, IBM Corporation, Armonk, NY, USA).

3 Results

3.1 Cytotoxic activities of extracts

Cytotoxic effects of methanol and water extracts obtained from *B. monnieri* and *C. demersum* on HepG2 and THLE2 cells were tested by XTT analysis. The maximum concentrations (400 µg/mL) of the extracts were the applications that reduced the viability of both cells the most. Accordingly, the most effective application on HepG2 cells was 400 µg/mL concentration of water extract of *B. monnieri*. The mentioned application reduced cell viability to 11.08%. At the same time, this application had statistically ($p < 0.05$) different data than all other applications. The application with the least effect on HepG2 cell viability (94.84%) was the 12.5 µg/mL concentration of methanol extract of *C. demersum* (Fig. 1).

When we look at the studies on THLE2 cells, the application that decreased cell viability (30.85%) the most was the highest concentration (400 µg/mL) water extract of *B. monnieri*. The application that had statistically ($p > 0.05$) no different data (32.01%) from the mentioned application was the maximum concentration of the water extract of *C. demersum*. The applications that showed the lowest viability activity on THLE2 cells were the lowest concentration of methanol and water extracts of *C. demersum* (97.55 and 97.74%, respectively). In addition, it was determined that these data were statistically ($p > 0.05$) indistinguishable from each other (Fig. 2).

IC_{50} data were used to determine the effective concentration values of extract applications. Accordingly, the most effective application on HepG2 cells turned out to be the water extract of *B. monnieri*, which has the lowest IC_{50} value (68.45 µg/mL) (Table 1). Similarly, the most effective application (IC_{50}: 127.05 µg/mL) on THLE2 cells was the water extract of *B. monnieri* (Table 2). Considering the highest IC_{50} values (173.35 and 228.46 µg/mL, respectively) occurring on HepG2 and THLE2 cells, the methanol extract of *C. demersum* appeared to have the lowest effect (Table 1 and 2).

3.2 Heatmap and cluster analyses

Heatmap and cluster analyses were used to determine the relationship levels between different extract applications applied on HepG2 and THLE2 cells. According to the heatmap analysis calculated by considering the IC_{50} values on HepG2 and THLE2 cells, the different color gradients of the methanol and water extracts of *C. demersum* were remarkable. These results were particularly important in terms of the high cytotoxic potential of *C. demersum* on cancer cells, although it showed lower cytotoxic effects on normal cells (Fig. 3a).

When we look at the cluster analysis performed to support the heatmap analysis, the methanol extract of *C. demersum* formed a separate cluster on both cells. The other three extract applications were included in a separate group under Cluster 2. All these results showed that the methanol extract of *C. demersum* has a cytotoxic effect on cancerous liver cells with low side effects (Fig. 3b and c).

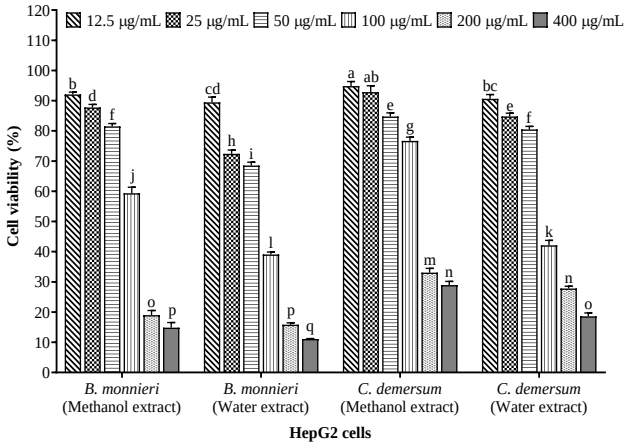

Fig. 1 Viability rates in HepG2 cells treated with different extracts from the plants (mean±standard deviation, $n = 3$) (Values indicated by different letters differ from each other at the level of $p < 0.05$.

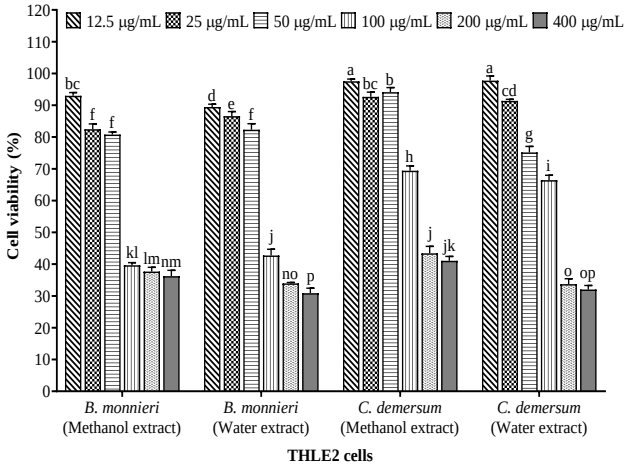

Fig. 2 Viability rates in THLE2 cells treated with different extracts from the plants (mean±standard deviation, $n = 3$) (Values indicated by different letters differ from each other at the level of $p < 0.05$.

Table 1 IC_{50} values (µg/mL) of extracts obtained from *B. monnieri* and *C. demersum* for cytotoxicity on HepG2 cells

Treatment	IC_{50} (Limits)	Slope ± Standard error (Limits)
B. monnieri (Methanol extract)	105.81 (97.29–115.39)	1.89 ± 0.07 (1.73–2.04)
B. monnieri (Water extract)	68.45 (62.61–74.82)	1.71 ± 0.07 (1.56–1.85)
C. demersum (Methanol extract)	173.35 (156.90–193.08)	1.71 ± 0.07 (1.55–1.86)
C. demersum (Water extract)	100.78 (91.90–110.83)	1.65 ± 0.07 (1.51–1.79)

Table 2 IC_{50} values (μg/mL) of extracts obtained from *B. monnieri* and *C. demersum* for cytotoxicity on THLE2 cells

Treatment	IC_{50} (Limits)	Slope ± Standard error (Limits)
B. monnieri (Methanol extract)	135.89 (120.83–154.26)	1.30 ± 0.06 (1.16–1.43)
B. monnieri (Water extract)	127.05 (113.85–142.79)	1.39 ± 0.06 (1.25–1.52)
C. demersum (Methanol extract)	228.46 (204.04–259.15)	1.63 ± 0.08 (1.47–1.79)
C. demersum (Water extract)	157.62 (142.54–175.62)	1.62 ± 0.07 (1.47–1.77)

4 Discussion

The results of this study provide significant insights into the cytotoxic effects of *B. monnieri* and *C. demersum* extracts on HepG2 cancer cells and THLE2 normal liver cells. The XTT analysis demonstrated that the water extract of *B. monnieri* exhibited the strongest cytotoxic activity on HepG2 cells, reducing cell viability to 11.08% at the maximum concentration of 400 μg/mL. This finding aligns with prior research, which highlights *B. monnieri*'s bioactive compounds, such as bacosides, that have been shown to induce apoptosis in cancer cells and inhibit tumor growth (Das et al. 2016; Smith et al. 2018; Aithal and Rajeswari, 2019). Moreover, the low IC_{50} value (68.45 μg/mL) further reinforces its potential as a promising therapeutic candidate for hepatocellular carcinoma treatment (Janani et al. 2010; Shefin et al. 2016).

Interestingly, the water extract of *B. monnieri* also demonstrated pronounced cytotoxicity on THLE2 cells, reducing viability to 30.85%, with a similarly low IC_{50} value (127.05 μg/mL). Although this suggests broad cytotoxic activity, it also raises concerns about its selectivity, as the effect on non-tumorous cells could limit its therapeutic window. This underscores the need for further investigation into *B. monnieri*'s selective toxicity to reduce potential side effects on healthy tissues (Ray et al. 2021; Malabadi et al. 2024). Recent findings have underscored *B. monnieri*'s pro-oxidant effects at high concentrations, which may contribute to its broad cytotoxicity, including potential effects on non-tumorous cells like THLE2 (Jyoti et al. 2007; Anand and Khanum, 2018). This broad activity raises questions regarding selectivity and highlights the need for further research into modifying *B. monnieri* extracts or dosing regimens to minimize harm to healthy cells.

In contrast, *C. demersum* exhibited a distinct cytotoxic profile, with its methanol extract showing the least impact on both HepG2 (IC_{50}: 173.35 μg/mL) and THLE2 cells (IC_{50}: 228.46 μg/mL). These findings are notable, as they suggest that *C. demersum* methanol extract may possess lower overall cytotoxicity, particularly on normal liver cells, making it a candidate for further investigation as a selective anticancer agent. Previous studies have identified bioactive compounds in *C. demersum*, such as flavonoids and phenolic acids, which may contribute to its selective anticancer properties (Awati et al. 2021; Masłyk et al. 2024). The heatmap and cluster analyses supported this distinction, with the methanol extract forming a separate cluster, emphasizing its potential for selective action against cancer cells.

This differential cytotoxicity, especially in the context of *C. demersum*, raises the possibility that specific compounds within this plant may target cancer cells more selectively. Compounds such as luteolin, apigenin, and phenolic acids have been reported in *C. demersum* extracts and are known for their anti-inflammatory, antioxidant, and pro-apoptotic activities (Eliašová et al. 2021; Nguyen et al. 2023). The relatively low cytotoxicity observed on THLE2 cells suggests that these compounds may have a targeted mechanism of action, potentially inducing apoptosis in cancer cells while sparing normal cells. Further studies are required to investigate the molecular pathways involved and to isolate the specific compounds responsible for this effect.

In terms of the broader clinical relevance, both *B. monnieri* and *C. demersum* exhibit mechanisms that may complement existing HCC therapies, such as chemotherapy, radiofrequency ablation, and immunotherapy. Current HCC treatments are often limited by resistance development, high recurrence rates, and adverse effects on liver function (Bruix et al. 2019; Chen et al. 2020). Combining conventional therapies with these plant-based extracts could potentially enhance treatment efficacy while reducing toxicity. For instance, *B. monnieri*'s ROS-inducing properties could sensitize cancer cells to chemotherapy agents that work through oxidative stress, while *C. demersum*'s compounds could provide an anti-inflammatory effect, possibly reducing adverse inflammatory responses often observed with HCC treatments (Mishra et al. 2019; Masłyk et al. 2024).

This study highlights the therapeutic potential of *B. monnieri* and *C. demersum* extracts in treating hepatocellular carcinoma. However, there are limitations that need to be addressed in future research. First, while XTT analysis provides valuable information on cytotoxicity, further studies, including mechanistic investigations of apoptosis, cell cycle arrest, and potential molecular targets, are necessary to fully understand the anticancer effects of these extracts (Cheung et al. 2023; Patra et al. 2023). Second, in vivo studies should be conducted to confirm the efficacy and safety of these extracts in more complex biological systems.

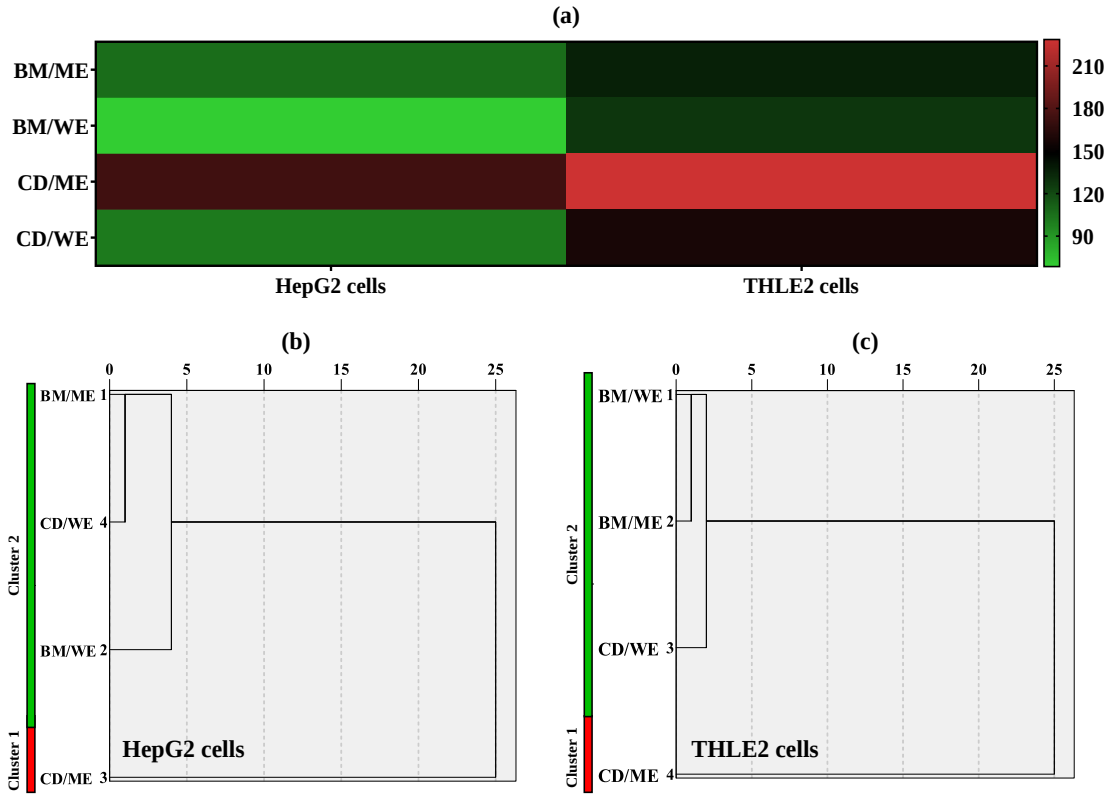

Fig. 3 (a) Heatmap based on IC_{50} values for cytotoxic activities of plant extracts and dendrogram (b) for HepG2 and (c) THLE2 cells (High and low activities were represented by red and green colour, respectively). BM/ME: Methanol extract of *B. monnieri*; BM/WE: Water extract of *B. monnieri*; CD/ME: Methanol extract of *C. demersum*; CD/WE: Water extract of *C. demersum*.

5 Conclusion

In conclusion, the study demonstrated that *B. monnieri* and *C. demersum* extracts exhibit significant cytotoxic effects on HepG2 liver cancer cells, with *B. monnieri* water extract being the most potent, reducing cell viability to 11.08%. Both plant extracts also showed varying degrees of cytotoxicity on non-tumorous THLE2 cells, with the water extract of *B. monnieri* having the most pronounced effect. However, the methanol extract of *C. demersum* displayed the lowest cytotoxicity on both cancerous and non-cancerous cells, suggesting its selective potential as a therapeutic agent with minimal side effects. Heatmap and cluster analyses further supported these findings, highlighting the distinct behavior of the *C. demersum* methanol extract, which formed a unique cluster, indicating its promising role in targeting cancer cells while sparing healthy ones.

Authors' contributions: B.E. designed the experiments. M.A. carried out the experiments. M.A., B.E., M.D. analysed the data and wrote the manuscript.

Conflict of interest disclosure:

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by Scientific Research Projects Commission of the Karamanoğlu Mehmetbey University (Project Number: 11-YL-23). This article is derived from Mustafa Arı's master's thesis, conducted under the supervision of Bugrahan Emsen, with Muhammet Dogan serving as the co-supervisor.

References

Aithal MGS, Rajeswari N (2019). Bacoside a induced sub-G0 arrest and early apoptosis in human glioblastoma cell line U-87 MG through notch signaling pathway. Brain Tumor Res Treat 7(1):25–32.

Anand T, Khanum F (2018). Attenuation of cytotoxicity induced by tBHP in H9C2 cells by Bacopa monniera and bacoside A. Pathophysiology 25(2):143–149.

Awati SS, Singh SK, Wadkar KA (2021). In vitro antioxidant potential and anticancer activity of *Ceratophyllum demersum* Linn. extracts on HT-29 human colon cancer cell line. Res J Pharm Technol 14(1):28–36.

Bai L, Ni L, Lu J, Zhang YY, Yin Y, Zhang W, Duan X (2024). Relationship between nausea and vomiting and physical activity in patients with lung cancer undergoing first chemotherapy. Front Oncol 14:1396637.

Brimson JM, Brimson S, Prasanth MI, Thitilertdecha P, Malar DS, Tencomnao T (2021). The effectiveness of *Bacopa monnieri* (Linn.) Wettst. as a nootropic, neuroprotective, or antidepressant supplement: analysis of the available clinical data. Sci Rep 11(1):596.

Bruix J, da Fonseca LG, Reig M (2019). Insights into the success and failure of systemic therapy for hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 16(10):617–630.

Chen Z, Xie H, Hu M, Huang T, Hu Y, Sang N, Zhao Y

(2020). Recent progress in treatment of hepatocellular carcinoma. Am J Cancer Res 10(9):2993.

Cheung AH, Hui CH, Wong KY, Liu X, Chen B, Kang W, To KF (2023). Out of the cycle: impact of cell cycle aberrations on cancer metabolism and metastasis. Int J Cancer 152(8):1510–1525.

Das DN, Naik PP, Nayak A, Panda PK, Mukhopadhyay S, Sinha N, Bhutia SK (2016). *Bacopa monnieri*-induced protective autophagy inhibits benzo[a]pyrenemediated apoptosis. Phyther Res 30(11):1794–1801.

Dogan M, Emsen B (2018). Anti-cytotoxic-genotoxic influences of in vitro propagated *Bacopa monnieri* L. Pennell in cultured human lymphocytes. Eurasian J Biol Chem Sci 1(2):48–53.

Dogan M, Ugur K (2024). Enhancing the phytoremediation efficiency of *Bacopa monnieri* (L.) Wettst. using LED lights: a sustainable approach for heavy metal pollution control. Environ Sci Pollut Res 31:53270–53290.

Dutta S, Mahalanobish S, Saha S, Ghosh S, Sil PC (2019). Natural products: An upcoming therapeutic approach to cancer. Food Chem Toxicol 128:240–255.

Eliašová A, Hrivnák R, Štefánová P, Svitok M, Kochjarová J, Oťaheľová H, Novikmec M, Pal'ove-Balang P (2021). Effects of ammonium levels on growth and accumulation of antioxidative flavones of the submerged macrophyte *Ceratophyllum demersum*. Aquat Bot 171:103376.

Emsen B, Dogan M (2018). Evaluation of antioxidant activity of in vitro propagated medicinal *Ceratophyllum demersum* L. extracts. Acta Sci Pol Cultus 17(1):23–33.

Emsen B, Sadi G, Bostanci A, Aslan A (2021). In vitro evaluation of cytotoxic, oxidative, genotoxic, and apoptotic activities of physodic acid from *Pseudevernia furfuracea* in HepG2 and THLE2 cells. Plant Biosyst 155(6):1111–1120.

Engloner AI, Németh K, Kós PB, Meglécz E, Bereczki J (2023). Genetic diversity of the submerged macrophyte *Ceratophyllum demersum* depends on habitat hydrology and habitat fragmentation. Front Plant Sci 14:1277916.

Esmeeta A, Adhikary S, Dharshnaa V, Swarnamughi P, Maqsummiya ZU, Banerjee A, Pathak S, Duttaroy AK (2022). Plant-derived bioactive compounds in colon cancer treatment: an updated review. Biomed Pharmacother 153:113384.

Fatima U, Roy S, Ahmad S, Ali S, Elkady WM, Khan I, Alsaffar RM, Adnan M, Islam A, Hassan MI (2022). Pharmacological attributes of *Bacopa monnieri* extract: current updates and clinical manifestation. Front Nutr 9:972379.

Flores JE, Thompson AJ, Ryan M, Howell J (2022). The global impact of hepatitis B vaccination on hepatocellular carcinoma. Vaccines 10(5):793.

Garcia-Oliveira P, Otero P, Pereira AG, Chamorro F, Carpena M, Echave J, Fraga-Corral M, Simal-Gandara J, Prieto MA (2021). Status and challenges of plant-anticancer compounds in cancer treatment. Pharmaceuticals 14(2):157.

García-Pras E, Fernández-Iglesias A, Gracia-Sancho J, Pérez-del-Pulgar S (2021). Cell death in hepatocellular carcinoma: pathogenesis and therapeutic opportunities. Cancers (Basel) 14(1):48.

Ghosh S, Khanam R, Acharya Chowdhury A (2021). The evolving roles of *Bacopa monnieri* as potential anti-cancer agent: a review. Nutr Cancer 73(11–12):2166–2176.

Granata V, Grassi R, Fusco R, Belli A, Cutolo C, Pradella S, Grazzini G, La Porta M, Brunese MC, De Muzio F (2021). Diagnostic evaluation and ablation treatments assessment in hepatocellular carcinoma. Infect Agent Cancer 16:1–22.

Haque MI, Singh PK, Ghuge S, Kumar A, Rai AC, Kumar A, Modi A (2022). A general introduction to and background of plant tissue culture: past, current, and future aspects. In: Advances in Plant Tissue Culture. Elsevier, pp 1–30

Jain S, Rathod M, Mishra G, Kumari V, Baksh H, Saxena S, Prasad L (2023). Plant tissue culture for medical therapy: unlocking the potential of medicinal plants. Curr J Appl Sci Technol 42(46):7–22.

Janani P, Sivakumari K, Geetha A, Ravisankar B, Parthasarathy C (2010). Chemopreventive effect of bacoside A on N-nitrosodiethylamine-induced hepatocarcinogenesis in rats. J Cancer Res Clin Oncol 136:759–770.

Jyoti A, Sethi P, Sharma D (2007). *Bacopa monniera* prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. J Ethnopharmacol 111(1):56–62.

Kok O, Emsen B, Surmen B (2023). Screening of in vitro cytotoxicity and antioxidant potential of selected endemic plants in Turkey. J Taibah Univ Sci 17(1):2217369.

Malabadi RB, Sadiya MR, Kolkar KP, Mammadova SS, Chalannavar RK, Baijnath H (2024). Role of plant derived-medicine for controlling cancer. Int J Sci Res Arch 11(1):2502–2539.

Masłyk M, Lenard T, Olech M, Martyna A, Poniewozik M, Boguszewska-Czubara A, Kochanowicz E, Czubak P, Kubiński K (2024). *Ceratophyllum demersum* the submerged macrophyte from the mining subsidence reservoir Nadrybie Poland as a source of anticancer agents. Sci Rep 14(1):6661.

Min Y, Wei X, Xia X, Wei Z, Li R, Jin J, Liu Z, Hu X, Peng X (2023). Hepatitis B virus infection: an insight into the clinical connection and molecular interaction between hepatitis B virus and host extrahepatic cancer risk. Front Immunol 14:1141956.

Mishra S, Dwivedi S, Mallick S, Tripathi RD (2019). Redox homeostasis in plants under arsenic stress. In: Redox Homeostasis in Plants. Springer, pp 179–198

Mishra SR, Behera BP, Singh VK, Mahapatra KK, Mundkinajeddu D, Bhat D, Minz AM, Sethi G, Efferth T, Das S (2024). Anticancer activity of *Bacopa monnieri* through apoptosis induction and mitophagy-dependent NLRP3 inflammasome inhibition in oral squamous cell carcinoma. Phytomedicine 123:155157.

Murashige T, Skoog F (1968). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 50(1):151–159.

Nguyen DTC, Van Tran T, Nguyen TTT, Nguyen DH,

Alhassan M, Lee T (2023). New frontiers of invasive plants for biosynthesis of nanoparticles towards biomedical applications: a review. Sci Total Environ 857:159278.

Patra D, Bhavya K, Ramprasad P, Kalia M, Pal D (2023). Anti-cancer drug molecules targeting cancer cell cycle and proliferation. Adv Protein Chem Struct Biol 135:343–395.

Ray A, Gulati K, Rehman S, Rai N, Anand R (2021). Role of nutraceuticals as adaptogens. In: Nutraceuticals. Elsevier, pp 229–244

Ruiz-Margáin A, Román-Calleja BM, Moreno-Guillén P, González-Regueiro JA, Kúsulas-Delint D, Campos-Murguía A, Flores-García NC, Macías-Rodríguez RU (2021). Nutritional therapy for hepatocellular carcinoma. World J Gastrointest Oncol 13(10):1440.

Saxena MK, Singh N, Kumar S, Mp D, Datta S (2021). Potent pharmaceutical products from aquatic plants–review. Asian J Pharm Clin Res 14(1):48–63.

Schlachterman A, Craft Jr WW, Hilgenfeldt E, Mitra A, Cabrera R (2015). Current and future treatments for hepatocellular carcinoma. World J Gastroenterol 21(28):8478.

Sharma AN, Dewangan HK, Upadhyay PK (2024). Comprehensive review on herbal medicine: emphasis on current therapy and role of phytoconstituents for cancer treatment. Chem Biodivers 21(3):e202301468.

Shefin B, Sreekumar S, Biju CK (2016). Identification of lead molecules with anti-hepatitis B activity in *Bacopa monnieri* (L.) Wettst. and *Cassia fistula* L. through in silico method. J Pharm Biol Sci 11(5):16–21.

Smith E, Palethorpe HM, Tomita Y, Pei J V, Townsend AR, Price TJ, Young JP, Yool AJ, Hardingham JE (2018). The purified extract from the medicinal plant *Bacopa monnieri*, bacopaside II, inhibits growth of colon cancer cells in vitro by inducing cell cycle arrest and apoptosis. Cells 7(7):81.

Stroffolini T, Stroffolini G (2023). A historical overview on the role of hepatitis B and C viruses as aetiological factors for hepatocellular carcinoma. Cancers (Basel) 15(8):2388.

Yazbeck V, Alesi E, Myers J, Hackney MH, Cuttino L, Gewirtz DA (2022). An overview of chemotoxicity and radiation toxicity in cancer therapy. Adv Cancer Res 155:1–27.

Zuzarte M, Salgueiro L, Canhoto J (2024). Plant tissue culture: industrial relevance and future directions. In: Plants as Factories for Bioproduction: Recent Developments and Applications. Springer, pp 1–15