KSÜ Tarım ve Doğa Derg 27 (5), 1026-1031, 2024
KSU J. Agric Nat 27 (5), 1026-1031, 2024
DOI:10.18016/ksutarimdoga.vi.1420650

Investigation (In-Vitro) of Antiproliferative Properties of *Pseudopediastrum boryanum* (Turpin) E. Hegewald Extracts in Various Cancer Cell Lines

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ABSTRACT

In this study, the antiproliferative effects of *Pseudopediastrum boryanum* isolated and cultured from Tokat Yeşilırmak River benthic habitats were investigated with in-vitro experiments. The Anticancer activity of algae extracts was tested in cancer cell lines using the BrdU cell proliferation ELISA method. Vero (African Green Monkey Kidney Tumour Cells) and HeLa (Human Uterine Carcinoma Cells) were examined in concentrations of 100, 250 and 500 µg mL^{-1}. As a result of antiproliferative tests, *P. boryanum* was found to have effective anti-cancer properties. *P. boryanum* extract concentrations of 250 and 500 µg mL^{-1} had a significant cytotoxic effect on Vero and HeLa cell lines after 24 hours. According to the results, *P. boryanum* is estimated to be a good option for quantitative pharmaceutical analyses and could be evaluated in the pharmaceutical industry.

Microbiology

Research Article

Article History
Received : 16.01.2024
Accepted : 28.03.2024

Keywords
Antiproliferative effect
BrdU
Chlorophyta
Pseudopediastrum boryanum

Pseudopediastrum boryanum (Turpin) E. Hegewald Ekstraktlarının Çeşitli Kanser Hücre Hatlarında Antiproliferatif Özelliklerinin (İn Vitro) Araştırılması

ÖZET

Bu çalışmada, Tokat Yeşilırmak Nehri bentik habitatlarından izole edilip kültürü yapılan *Pseudopediastrum boryanum*'un antiproliferatif etkileri, invitro deneylerle araştırıldı. Alg ekstraktlarının antikanser aktivitesi, BrdU hücre proliferasyon ELİSA yöntemi kullanılarak kanser hücre hatlarında denendi. Vero (Afrika Yeşil Maymun Böbrek Tümör Hücreleri) ve HeLa (İnsan Servikal Kanser Hücreleri) üzerinde 100, 250 ve 500 µg mL^{-1}' lik konsantrasyonlarda incelendi. Antiproliferatif testler sonucunda, *P. boryanum*'un, etkili antikanser özelliğe sahip olduğu görüldü. *P. boryanum*'un 250 ve 500 µg mL^{-1}' lik ekstrakt konsantrasyonlarının 24 saat sonunda Vero ve HeLa hücre hatları üzerinde, önemli derecede sitotoksik etki gösterdiği kaydedildi. Elde edilen sonuçlara göre, *P. boryanum'un* kantitatif farmasötik analizler için iyi bir aday olduğu ve ilaç endüstrisinde değerlendirilebileceği tahmin edilmektedir.

Mikrobiyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 16.01.2024
Kabul Tarihi : 28.03.2024

Anahtar Kelimeler
Antiproliferatif etki
BrdU
Chlorophyta
Pseudopediastrum boryanum

Atıf İçin : Demiriz Yücer, T. (2024). *Pseudopediastrum boryanum* (Turpin) E. Hegewald Ekstraktlarının Çeşitli Kanser Hücre Hatlarında Antiproliferatif Özelliklerinin (İn Vitro) Araştırılması. *KSÜ Tarım ve Doğa Derg 27* (5), 1026-1031. https://doi.org/10.18016/ksutarimdoga.vi.1420650

To Cite: Demiriz Yücer, T. (2024). Investigation (In-Vitro) of Antiproliferative Properties of *Pseudopediastrum boryanum* (Turpin) E. Hegewald Extracts in Various Cancer Cell Lines. *KSU J. Agric Nat 27*(5), 1026-1031. https://doi.org/10.18016/ksutarimdoga.vi.1420650

INTRODUCTION

Chlorophyta (Green algae) is a big and widespread group that can form dense populations in all aquatic environments. It also has species that have a complex structure, as well as single-celled species. They can be in the form of phytoplankton of microscopic size in the marine environment, as well as in meters in macroscopic size (Norris, 2010). Chlorophyta algae can also develop a symbiotic life with tropical plants, foraminifera, lichens.

This group, which has been worldwide in vast areas, has become widespread from the Antarctic to the Arctic, oceans or even freshwaters (Leliaert et al., 2007), and they are represented by 6801 species belonging to 13 classes at the macro and micro level (Guiry & Guiry, 2021).

Plant organisms that can produce their food by photosynthesis are called "primary producers" as they are always the first ring in the food chain in nature. About 70% of the oxygen in the atmosphere, which is the source of our lives, is produced by the photosynthesis of algae. Algae have many economic benefits. In addition to being used as human and animal food with high protein that they contain, they are also often used in the production of natural fertilizer, medicine, pharmacy, cosmetics, and food industries. Another reason why algae are among the most studied groups of organisms today is that they can be produced relatively quickly for other organisms in cultural environments (Altuner et al., 2002).

One of the most significant illnesses that are currently threatening humankind's future is cancer. There is no basic cause for cancer. In all types of cancer, some of the body's cells begin to divide without stopping and spread to adjacent tissues, causing uncontrolled cell growth throughout the body (Mofeed et al., 2018; Yousefi et al., 2018).

Currently, existing drugs used to treat cancer cause some side effects such as vomiting, diarrhea, fatigue, and nausea. Therefore, there have been no safe drugs to be used in cancer treatments. Thus, it's critical to find and classify novel, low-cost, safe, and less hazardous anticancer agents derived from natural sources. The majority of pharmacological drugs come from different plants, algae, and microorganisms (Mofeed, 2019).

This article aimed to investigate the antiproliferative effects of *P. boryanum*. The anticancer activity of algal extracts was tested in cancer cell lines using the BrdU cell proliferation ELISA method. Vero (African Green Monkey Kidney Tumor Cells) and HeLa (Human Uterine Carcinoma Cells) were examined at concentrations of 100, 250 and 500 µg mL^{-1}.

MATERIALS and METHODS

Isolation and culture of algae

P. boryanum was isolated in the laboratory using mechanical isolation and microinjection techniques after being removed from the Yeşilırmak River (Tokat). They were then placed in a culture environment at 26 °C (155 µmol/m2/h, L: D period) in a Sanyo MLR 351 climate cabin containing BG11 medium (Andersen, 2004; Pabuçcu & Demiriz Yücer, 2022). Purely cultured algal species were stored in a −86 °C freezer in a culture collection to be used for postharvest bioactivity studies (Rippka et al., 1979; Lobban et al., 1988; Coşkun et al., 2023).

Preparation of algae extracts

Algae extract was prepared according to the literature method (Chon et al. 2009). 5 g of dried and grounded algae material was soaked in 100 mL methanol for two days at room temperature. After filtration (Whatman 1 filter paper), the solvent was evaporated by reduced pressure to produce the extract (Erenler et al., 2016, Elmastaş et al., 2018).

Antiproliferative Activity Tests

Cell lines

P. boryanum was tested for its antiproliferative effect using two cancer cell lines: Vero (African Green Monkey kidney tumor cells) and HeLa (Human Cervical Cancer Cells).

Cell culture

Trypsin-EDTA (10 mL) was treated with DMEM medium-prepared cells for one to two minutes at 37°C in a CO_2 incubator. The environment was neutralized by adding 10 mL of DMEM medium to the flask removed from the incubator. After giving the flask a good shake, the cell solution was moved to the Falcon tube (600 rpm, 5 min), and centrifugation was used to precipitate the cells (Dulbecco & Freeman, 1959; Liu et al., 2002; Yang et al., 2004).

Proliferation experiment of in Vitro tumour cells (Vero, HeLa)

Stock solution of the samples, 5-florouracil (5-FU) and cisplatin were prepared in sterile dimethyl sulfoxide (DMSO) and were diluted with Dulbecco's modified eagle's medium (DMEM; 1:20). The final concentration of DMSO was kept below 1% in all tests. The stock solutions were stored at±4°C until usage. Sterile stock solutions of algae extract at three different concentrations (100, 250, and 500 µg mL^{-1}) were added to all wells except the control group, resulting in a total volume of 200 µL in the wells. Instead of the test substances, sterile solvent DMSO was added to the negative control wells and left the cells in incubation for 24 hours. Treated cells were incubated at 37°C with 5% CO_2 for 24 h. Cell proliferation was measured by using BrdU Cell Proliferation ELISA, a colorimetric immunoassay based on BrdU incorporation into the cellular DNA according to manufacturer's procedure. At the end of this period, BrdU cell ELISA was used in accordance with a manufacturer's protocol to examine an experiment on cellular proliferation and its Absorbance at wavelengths ranging from 450 nm to 650 nm has been measured using an ELISA reader (Masterson & O'Dea, 2007; Işıkdağ et al., 2011; Lehner et al., 2011).

Statistical analyses

The SPSS® program was used for statistical evaluations for anticancer activity studies. Differences between experimental groups were

evaluated statistically by applying one-way variance analysis (one-way ANOVA) and the Duncan test as post hoc (differences between the results were considered statistically significant at the level of $p<0.01$). The results were expressed as mean ± standard error.

RESULTS and DISCUSSION

Antiproliferative Activity

Results of proliferation experiment with Vero cells

In the analysis of variance, the difference between groups was found to be significant when the test results at concentrations of 100, 250 and 500 µg mL^{-1} ($p<0.01$) were considered. *P. boryanum* was found to have a higher effect than DMSO and the control group in terms of anticancer test results at 500 g mL and 250 g mL in the multiple comparison Duncan test ($p<0.01$). (Table 1, Figure 1).

Results of proliferation experiment with HeLa cells

According to the variance analysis results, the difference between groups was found to be significant when the test results at concentrations of 100, 250 and 500 µg mL^{-1} were considered ($p<0.01$). In terms of anticancer agent test results at concentrations of 250 and 500 µg mL^{-1} in the multiple comparison (Duncan) test, *P. boryanum* was found to be higher than DMSO and control group ($p<0.01$) (Table 2, Figure 2).

Table 1. Statistical analysis results of anticancer activity tests for Vero Cells
Çizelge 1. Vero Hücreleri için antikanser aktivite testlerinin istatistiksel analiz sonuçları

Vero Afrika Yeşil Maymun Böbrek Tümör Hücreleri Groups Gruplar	Again (N) Tekrar (N)	100 µg mL^{-1} x±(SS) ortalama±standart sapma	250 µg mL^{-1} x±(SS) ortalama±standart sapma	500 µg mL^{-1} x±(SS) ortalama±standart sapma
Control	3	2.561±0.171	2.561±0.171	2.561±0.171
DMSO	3	2.8440±0.040	2.808±0.0410	2.427±0.355
P. boryanum	3	2.723±0.103	2.554±0.0132	1.774±0.022

x±(SS); Value±standard deviation

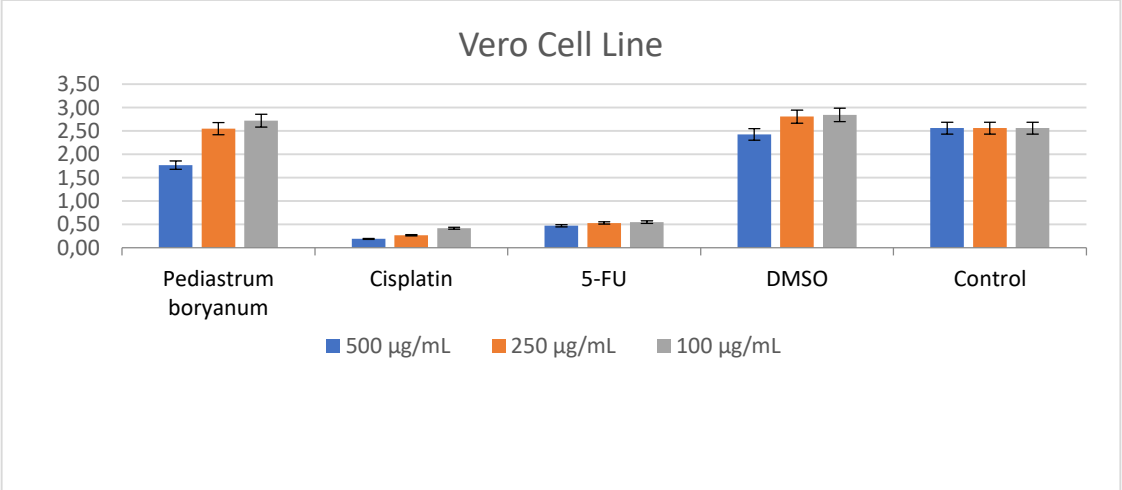

Figure 1. Results of antiproliferative activity on Vero Cells ($p<0.01$)
Şekil 1. Vero Hücreleri üzerindeki antiproliferatif aktivitenin sonuçları ($p<0.01$)
Cisplatin; Anticancer compound, 5-FU; Anticancer compound, DMSO; Dimethylsulfoxide

Table 2. Statistical analysis results of anticancer activity tests for HeLa Cells
Çizelge 2. HeLa Hücreleri için antikanser aktivite testlerinin istatistiksel analiz sonuçları

HeLa İnsan Servikal Kanser Hücreleri Groups Gruplar	Again (N) Tekrar (N)	100 µg mL^{-1} x±(SS) ortalama±standart sapma	250 µg mL^{-1} x±(SS) ortalama±standart sapma	500 µg mL^{-1} x±(SS) ortalama±standart sapma
Control	3	2.486±0.023	2.486±0.023	2.486±0.023
DMSO	3	2.030±0.030	1.896±0.045	1.240±0.045
P. boryanum	3	2.046±0.020	1.740±0.020	0.970±0.020

x±(SS); Value±standard deviation

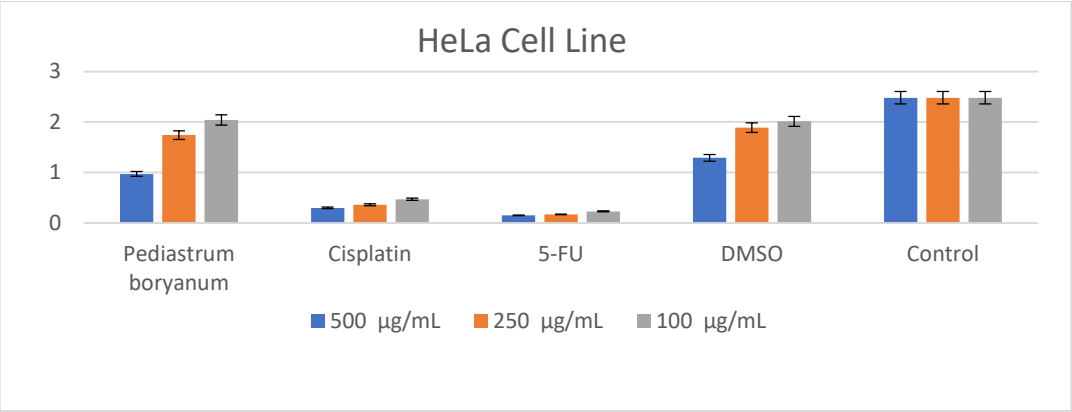

Figure 2. Anticancer activity results for HeLa Cells (p<0.01)
Şekil 2. HeLa Hücreleri için antikanser aktivite sonuçları (p<0.01)
Cisplatin; Anticancer compound, 5-FU; Anticancer compound, DMSO; Dimethylsulfoxide

Currently, the compounds derived from algae extracts with antimicrobial and antiproliferative activity have caught intense interest worldwide. Amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulfonates and fatty acid components are among the algal constituents. In addition, acrylic acid, especially found in some planktonic algae and responsible for antimicrobial activity, is also available (Mtolera & Semesi, 1996).

Among the functional components identified based on algae, natural pigments, especially decobili proteins and carotenoids, are notable. Because of the antibacterial and antioxidant effects of these substances, they are especially preferred in pharmaceutical applications. Natural pigments and algal photosynthesis and pigmentation play an important role in antioxidant metabolism and have a number of benefits such as anti-cancer, anti-inflammatory, anti-obesity, anti-angiogenesis and neuroprotective effects (Sathasima et al., 2017).

It was found in the study that the algae species used had different antiproliferative effects at different concentrations on the tested cancer cell lines. *P. boryanum*'s extract concentration of 250 and 500 µg mL^{-1} was found to have had a significant cytotoxic effect on Vero and HeLa cell lines after 24 hours. In particular, a high activity of cisplatin and 5-FU, an anticancer compound, was noted in the study (Figure 1, 2, Table 1, 2).

BG11 medium was used as the incubation medium in this study. This is the environment where P. boryanum shows the best biological activity of the culture. Garcez et al. (2020) examined the antioxidant effects of *P. boryanum* produced in 6 different cultural settings and found that they showed high amounts of biomass and phenolic compound when incubated in BG11 It has been stressed that if *P. boryanum* is grown in this medium, it may be a new source of polyphenols with potential antioxidative effects. (Garcez et al., 2020).

In Pediastrum taxa, various toxic effects against certain cells have been observed. For example, polyunsaturated fatty acid (hexadecatetraenoic acid) isolated from a Pediastrum species was found to have a toxic effect at a concentration of 25 µg/ml, preventing the development of fertilized Echinoderm eggs (Murakami et al., 1989). The toxicity of *P. boryanum* against HeLa and Vero cells has been shown in our study.

Some species of Pediastrum, on the other hand, were found to have helped a lot in moisturizing epithelial tissue in cosmetics. For example, Wang et al. (2015) stated that Pediastrum duplex can be used to moisturize skin. It has been unclear while *P. boryanum* has a similar effect.

Syahril et al., (2011) conducted a study on *Chlorella* sp. and *Spirulina* sp. and used the MTT method, which is a mitochondrial-based method, and human breast cancer cell line MCF-7, HepG2 human liver cancer cell line, and normal cell line WRL-68. It was found in the study that ethanol extract of *S. platensis* had an antiproliferative effect on human breast cancer cell line-MCF-7 at a concentration of 85 µg/mL for 72 hours but had no effect for 24 and 48 hours. In this study, DMSO-dimethyl sulfoxide extracts of *P. boryanum* were found to have a significant cytotoxic effect on Vero and HeLa cell lines for 24.

Yousefi et al. (2018) conducted research using *P. tenuis, C. sinuosa, I. stellate, D. indica* and used breast cancer cells, the most common cancer type in women. The extract obtained from *D. indica* has been shown to have a cytotoxic effect on breast cancer cell lines for 24 hours at a concentration of 50 g mL in a study conducted using the MTT method.

Alghazeer et al. (2018) researched Chlorophyta (*Ulva, Codium),* Phaeophyta (*Cystoseira, Sargassum*), and Rhodophyta (*Gelidium, Hypnea, Jania*), and used human colorectal carcinoma (Caco2) and human corneal epithelial cells (HCEC). Extracts of algae

species were examined using the MTT method at concentrations of 50, 100, 150, and 200 µg mL^{-1}. *C. crinita*, out of the examined species, was found to have a cytotoxic effect on the Caco-2 cancer cell line. According to the researchers, anticancer research programs could use marine algae rich in bioactive compounds.

CONCLUSION

The fact that algal products and their derivatives are compatible with the body's immune system and that they can help the body increase its resistance without damaging healthy cells when effective value is obtained are the main reasons why they are preferred in treatments. In the discovery of new alternatives to synthetic drugs, which have many side effects on human cells and also cause cell resistance, algae have been the main focus.

The increase in the resistance of microorganisms to existing antibiotics has led to an increase in the search for photosynthetic antibiotics and anticancer agents and caused an increase in the research on this subject.

In future studies, the antiproliferative effect of this algal species will be investigated with different cancer cell lines and at varying concentrations, and preliminary studies can be completed to move on to in vivo studies. With the current results, it can be said that 250 and 500 µg mL^{-1} concentrations of *P. boryanum* will be suitable for in vivo studies.

Based on this study, it is also possible to say that *P. boryanum* can be used as an alternative anticancer drug source in the healthcare field, especially in the pharmaceutical industry, in the future.

ACKNOWLEDGMENT

The author thanks Prof.Dr. Köksal Pabuçcu for his help in the diagnosis of algae species.

Author's Contributions

The experimental work in this article was done and written by a single author.

Conflict of Interest Statement

No conflict of interest is associated with this work.

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