

Studies on antioxidant, anticancer, DNA cleavage activity and GC-MS analysis of the ethanol extracts of *Ecballium elaterium* (L.) A. Rich., plant parts

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Anahtar Kelimeler

Ecballium elaterium,
Cucurbitacin,
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antikanser,
pBR322 DNA

Abstract: *Ecballium elaterium* is a native species of the south of Europe and the Mediterranean catchment, occasionally cultivated for its use as a medicinal plant. In this study, it was investigated to phytochemicals profiles, cucurbitacin content, antioxidant, DNA cleavage properties and anticancer activity of *E. elaterium* seed, fruit peel and juice ethanol extracts. Phytochemical components of all extracts were determined by the phytochemical analysis methods. Quantitative cucurbitacin content was determined by GC-MS, the free radical scavenging activity of the extracts was determined by the DPPH method, separation ability on pBR322 DNA was determined by agarose gel electrophoresis and cytotoxic effects were determined by MTT analysis on the HepG2 liver cancer line. Phytochemical and GC-MS analysis results indicate cucurbitacins containing glycoside molecules that can be evaluated as a drug in the structure of the plant. The highest cucurbitacin content of 3.73 % was found in *E. elaterium* fruit peel. DPPH activity of juice, fruit peel and seed extracts were determined to be 29.39%, 54.12%, and 79.45%, respectively at the highest concentration. All of the extracts effected on the superhelix structure of plasmid DNA. There was a decrease in the viability of HepG2 liver cancer cells due to the increased concentration. After 48 hours of incubation, IC₅₀ values for juice, fruit peel and seed extracts were determined as 309.02 µg/ml, 525.43 µg/ml and 549.54 µg/ml, respectively. It seems that *E. elaterium* juice, fruit peel and seed extracts have promising potential as future natural anticancer agents in the food industry..

Ecballium elaterium (L.) A. Rich bitki parçalarının etanol ekstraktlarının antioksidan, antikanser, DNA ayırma aktivitesi ve GC-MS analizi üzerine çalışmalar

Keywords

Ecballium elaterium,
Kukurbitasin,
antioksidan,
antikanser,
pBR322 DNA

Öz. *Ecballium elaterium*, Avrupa'nın güneyinde ve Akdeniz havzasında bulunan yerli bir türdür ve bazen tıbbi bitki olarak kullanılmak üzere yetiştirilir. Bu çalışmada *E. elaterium* tohumu, meyve kabuğu ve meyve suyu etanol ekstraktlarının fitokimyasal profilleri, cucurbitacin içeriği, antioksidan, DNA ayırma özelliği ve antikanser aktiviteleri araştırıldı. Tüm ekstraktların fitokimyasal bileşenleri fitokimyasal analiz yöntemleriyle gerçekleştirildi. Kantitatif kukurbitasin içeriği GC-MS, ekstraktların serbest radikal temizleme aktivitesi DPPH yöntemiyle, pBR322 DNA üzerindeki ayırma özelliği agaroz jel elektroforezi ve sitotoksik etkileri HepG2 karaciğer kanseri hattı üzerinde MTT analizleri ile belirlendi. Fitokimyasal ve GC-MS analiz sonuçları bitkinin yapısında ilaç olarak değerlendirilebilecek glikozit molekülleri içeren kukurbitasinlerin varlığına işaret etmektedir. En yüksek cucurbitacin içeriği %3,73 ile *E. elaterium* meyve kabuğunda bulunmuştur. Meyve suyu, meyve kabuğu ve tohum ekstraktlarının DPPH aktivitesinin en yüksek konsantrasyonda sırasıyla %29,39, %54,12 ve %79,45 olduğu belirlendi. Ekstraktların tümü plazmid DNA'nın

süperheliks yapısı üzerinde etkili olmuştur. Konsantrasyon artışına bağlı olarak HepG2 karaciğer kanseri hücrelerinin canlılığında azalma meydana gelmiştir. 48 saatlik inkübasyonun ardından meyve suyu, meyve kabuğu ve tohum ekstraktlarının IC50 değerleri sırasıyla 309,02 µg/ml, 525,43 µg/ml ve 549,54 µg/ml olarak belirlendi. *E. elaterium* meyve suyu, meyve kabuğu ve tohumunun gıda endüstrisinde gelecekteki doğal antikanser ajanları olarak umut verici bir potansiyele sahip olduğu görülmektedir.

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1. Introduction

Plants are one of the most important sources of natural remedies used in traditional therapy methods. Since the bioactive substances in plants are very rich and effective, the variety of plants that can be used as herbal medicine has increased rapidly between scientific environments. Medicinal plants and herbal medicines are widely used in traditional cultures worldwide, and are becoming increasingly popular as natural options to artificial chemicals in the modern lifestyle [1].

Phytochemicals, which are useful in the treating of some diseases with their individual or synergistic effects, are of vital in developing of new drugs and preparing therapeutic agents in the pharmaceutical industry. [2,3]. Screening of plant extracts, which are natural sources, in the developing of new drugs, is a new approach to finding therapeutically active ingredients. [4,5]. Phytochemicals such as flavonoids, tannins, saponins, alkaloids and terpenoids have many biological properties, including antioxidant, anti-inflammatory, anti-diarrheal, anti-ulcer and anticancer activities. [5].

Ecballium elaterium (L.) A. Rich, known as 'squirting cucumber' from the Cucurbitaceae family, is abundant in the Mediterranean region and its fruit juice is used by the public in to treat of sinusitis in Anatolia [6]. All cucurbit species produce a class of triterpenoids known as cucurbitacins. Cucurbitacins, derived from the cucurbitane skeleton, are a group of tetracyclic triterpenoids found mainly in the Cucurbitaceae family. Triterpenoids, found in free or glycosidic form, give the plant a hot taste and are probably produced as a defense mechanism against pests [7]. These molecules are known to possess a broad spectrum of pharmacological activities such as anti-inflammatory, antipyretic, anticancer, analgesic, antimicrobial, antitumor, immunomodulatory, and hepatoprotective activities [8-11].

The aim of our study is to determine the cytotoxic effects of *E. elaterium* fruit peel, seed and juice by detecting the phytochemical content, especially cucurbitacins, using GC-MS analysis. Also, in study the antioxidant activity of the plant extract and its cleavage effect on pBR322 plasmid DNA were investigated. Our study will contribute to the developing new strategies for using of herbal resources to prevent, prevent, delay or cure cancer.

2. Material and Method

2.1. Plant extraction and fractionation

E. elaterium plant samples were collected from Istanbul province between October and November 2019. Fruit peel, juice and seed parts of the plant were used. Seed, fruit peel and juice ethanol extracts of *E. elaterim* were prepared according to the method of Adwan et al. (2011) [11].

Fruits peels were homogenized and dried in an incubator at 37 °C. Exposure to light was avoided to prevent loss of effective ingredients. The samples were then mixed well with magnetic stirrer in 80% ethanol for 24 hours at room temperature. The insoluble materials were centrifuged at 10000 rpm at 4 °C for 10 minutes. Then the liquid was evaporated in the oven at 37 °C.

Seed samples were dried at room temperature before extraction, and the same process was performed. The juice was drawn directly, mixed in 80% ethanol and left for one day at room temperature. Centrifugation was then carried out at 10000 rpm for 10 minutes at 4 °C. Extracts were kept in the refrigerator at 4 °C.

2.2. GC-MASS analysis of *E. elaterium* extracts

Gas chromatography-mass spectrometry (GC/MS) was used for quantitatively analyze of *E. elaterium* seeds, fruit and juice ethanol extracts. The analysis was performed using a GC (SHIMADZU 2010 Plus) interfaced with a mass select or detector (MSD, SHIMADZU QP2010 Ultra) equipped with a polar Rtx-5ms (5% phenylmethylpolysiloxane) capillary column (30 m × 0.25 mm id and 0.25 µm film thickness). Helium with a linear velocity of 1.15 ml/min was used as the carrier gas. The components were identified by comparing the mass spectra and retention times with those of the organs and the computer mapping with the NIST and WILEY library, and the fragmentation model of mass spectral data. GC/ MS analysis was done in Erciyes University Technology Research and Application Center (TAUM).

2.3. Phytochemical analysis of *E. elaterium* extracts

To obtain more details about the composition of *E. elaterium*, the following qualitative analyses were made according to the Teli et al. (2017) method [12].

Saponin and Tannin Test: 1 ml extract was placed in the test tube and vigorously mix with vortex. If foam formation is observed in the tube, the presence of saponin in the extract will be determined.

Phenol and Tannin Test: By adding 2 ml of 2% FeCl₃ solution onto 1 ml of extract and mix, if the formation of a blue-green or black color in the mixture is observed. The formation of these colors indicates the presence of phenol and tannin in the extract.

Terpenoids Test: 2 ml of chloroform and 2 ml of concentrated sulfuric acid were mixed on 1 ml of plant extract. Formation of a red intermediate phase indicates the presence of terpenoids in the extract.

Flavonoids Test: 1 ml extract was mixed with some zinc powder and concentrated hydrochloric acid was added drop by drop. The observation of a red color after a few minutes indicates the presence of flavonoids in the extract.

Glycoside Test: The mixture obtained by adding 2 drops of 2 % FeCl₃ to 1 ml of glacial acetic acid was mixed with to 1 ml of extract and transferred to the tube containing 2 ml of concentrated sulfuric acid. Formation of a brown ring in the intermediate phase indicates the presence of glycoside in the extract.

2.4. Antioxidant activity determination of *E. elaterium* extracts

The antioxidant activity of *E. elaterium* fruit peel, seed and juice extracts was evaluated using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The method is based on the measurement of the colour change in the characteristic purple colour of the radical using a spectrophotometer at a wavelength of 517 nm [13] Briefly, dilutions of 30, 15, 7.5, 3.75, 1.87, 0.93, 0.46 and 0.23 mg/ml concentrations of *E. elaterium* fruit peel, seed and juice extracts were prepared and 50 µl of these dilutions were taken into test tubes. After adding 1 ml of 0.1 mM DPPH radical prepared with methanol, it was mixed with vortex and keep at room temperature for 2 hours in the dark. The same process was repeated simultaneously for a blind tube containing solvent instead of compound. The absorbance values of the mixtures were read on a 517 nm wavelength in spectrophotometer. Measurements were repeated three times for each compound and the results averaged. The free radical scavenging effect of the solutions is indicated as % inhibition of DPPH absorption.

This value is calculated according to the formula:

Radical removal activity (% Inhibition) = $[100 \times (A \text{ control} - A \text{ sample}) / A \text{ control}]$.

IC₅₀ values (indicating the concentration of the extract (mg/mL) providing 50% radical scavenging) were calculated from the graph plotted scavenging percentage against extract concentration.

2.5. DNA cleavage activity on pBR322 plasmid DNA of *E. elaterium* extracts

Agarose gel electrophoresis was used to observe the effects of *E. elaterium* extract on pBR322 plasmid DNA [14]. 3 µl plasmid DNA (0.5 µg / ml) was added over 30 µl of fruit peel, juice and seed ethanol extracts prepared at different concentrations (100, 50, 25, 12.5 and 6.25 ppm). Samples were incubated at 37 °C for 24 hours in a dark place. After incubation, 10 µl of the sample-DNA mixture was mixed with loading buffer and loaded into 1% agarose gel, and electrophoresis was performed for 2 hours at 60 V in TAE buffer. After electrophoresis, gels were stained with ethidium bromide (0.5 µg/ml) and photographs were taken in the imaging system. Experiments were

done in three replications.

2.6. Cytotoxic effect of *E. elaterium* ethanol extracts

In order to assess the cytotoxicity of *E. elaterium* the MTT assay was used [15]. We tested ethanol extracts of plant on HepG2 (Human, Liver cancer cell line, Epithelial-like, hepatocellular carcinoma) cell line. The number of cells required for the test was calculated and the cells were cultured until they reached this number. Cells were cultured on high glucose DMEM (Sigma, USA) medium containing 10% FBS (Biological Industries, USA), 1% L-glutamine (Biological Industries, USA) and 1% Penicillin Streptomycin (Biological Industries, USA). When the cells reached a sufficient number, they were washed two times with PBS (Biological Industries, USA) and incubated with 0.05% Trypsin-EDTA (Biological Industries, USA) for 5 minutes to leave the flask. Cells taken from the flask after incubation were placed in sterile conical-based centrifuge tubes and centrifuged at 350xg for 5 minutes. The cell pellet obtained was resuspended again with the culture medium.

Cell numbers were then calculated and cultivated in 96-well culture dishes based on "F", where MTT testing would be performed, with 3×10^3 cells per well. Culture medium was added to each well with 200 μ l and incubated in a 37 °C CO₂ incubator. The next day, the cells were examined under an inverted light microscope, when the cells were found to be adherent, plant extracts of different concentrations (1000, 500, 250, 125, 62.5, 31.25 μ g/ml) were added to the cells. Extracts were incubated with cells for 48 hours. At the end of 48 hours, MTT salt was prepared with PBS and after sterilization it was added to the cells so that the final concentration was 0.5 mg/ml. Cells incubated with MTT for 4 hours were examined under an inverted light microscope and the medium on the cell was carefully aspirated when the formation of formazan crystals was seen. Then 100 μ l of DMSO was added to each well and incubated in the dark to dissolve the formazan crystals formed for 15 minutes. At the end of 15 minutes, 96-well culture vessels were read at 560 nm in an ELISA reader (Promega, Glomax) to read the purple color formed by dissolving formazan crystals. Each experiment was performed in 3 replications as an independent set. As the negative control in the experimental setup, plant extracts were not added to the determined wells. While the absorbance of the cells used in the negative control is considered 100% alive, the ratio of the absorbance in other wells to this absorbance gave the percent viability of the cells in that well.

3. Results and Discussion

3.1. GC-MS analyses the results of extracts

GC-MS showed chromatograms obtained as a result of the cucurbitacin content of *E. elaterium* seed, fruit peel and fruit juice ethanol extracts. As a result of scanning the analysis results in the WILEY database, the names of the possible main and other compounds in the Table 1. According to this screening result, Cucurbitacin B as the main compound has been detected in fruit peel and fruit juice extracts, but not in seed extract. Other substantial compounds detected in all extracts: Saturated fatty acids, unsaturated fatty acids, fatty esters, glycosides, diterpenoids, diterpene, glycerolipids and organic compounds.

Table 1. Bioactive compounds found in ethanol extract of *E. elaterium* seed, fruit peel and juice

Compound Name	Molecular formula	Classification	RT Time			Field%		
			Fruit Peel	Seed	Juice	Fruit Peel	Seed	Juice
Ethyl alpha-d-glucopyranoside	C ₈ H ₁₆ O ₈	Glycosides	11.7			37.28		
Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	Saturated fatty acid	13.1		13.1	2.65		0.84
Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	Saturated fatty acid	14.2			0.99		
Hexadecanoic acid, methyl ester (Methyl palmitate)	C ₁₇ H ₃₄ O ₂	fatty acid methyl ester	14.8			0.84		0.26

n-Hexadecanoic acid (Palmitic Acid)	C ₁₆ H ₃₂ O	Saturated fatty acid	15.2	15.2		17.73	30.61	19.42
Hexadecanoic acid, ethylester (Ethyl palmitate)	C ₁₈ H ₃₆ O ₂	fatty acid ethyl ester	15.5	15.5		3.71	3.23	1.84
9-Octadecanoic acid, methylester (Elaidic acid)	C ₁₉ H ₃₆ O ₂	unsaturated trans fatty acid	16.6			8.47		
Methyl stearate	C ₁₉ H ₃₆ O ₂	fatty acid methylester	16.8			1.77		
Octadecanoic acid (stearic acid)	C ₁₈ H ₃₆ O ₂	Saturated fatty acid	17.2	17.2		5.22	13.00	
Cucurbitacin B, dihydro -	C ₃₂ H ₄₈ O ₈	Triterpenoids	19.3		20.5	3.73		1.54
1-Heptacosanol	C ₂₇ H ₅₆ O	primary fatty alcohol	21.3			13.76		
9-Octadecenoic acid (Z)	C ₁₈ H ₃₄ O ₂	unsaturated fatty acid		14.2	14.2		1.11	0.51
Heptadecanoic acid	C ₁₇ H ₃₄ O ₂	saturated fatty acid		16.2	16.2		1.19	0.61
9,12-Octadecadienoic acid (Z, Z)	C ₁₈ H ₃₂ O ₂	unsaturated fatty acid		17.0	17.0		7.89	3.24
9,12,15-Octadecatrienoic acid, (Z, Z, Z)	C ₃₆ H ₆₀ O ₄	unsaturated fatty acid		17.1	17.0		14.13	11.50
Octadecanoic acid, ethylester	C ₂₀ H ₄₀ O ₂	fatty acid ethyl ester		17.5			4.20	
Hexadecanoic acid, 2- hydroxy-1- (hydroxymethyl) ethyl ester	C ₁₉ H ₃₈ O ₄	Fatty esters		20.3			6.24	
Octadecanoic acid, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₂ O ₄	Fatty esters		22.1	22.2		1.66	11.95
Oxalic acid, cyclohexylpentyl ester	C ₁₃ H ₂₂ O ₄	Fatty esters			5.1			3.17
n-Decanoic acid	C ₁₀ H ₂₀ O ₂	Unsaturated fatty acids			8.0			2.90
Apocynin	C ₉ H ₁₀ O ₃	Phenolic acids			9.8			0.33
Undecanoic acid	C ₁₁ H ₂₂ O ₂	Unsaturated fatty acids			10.7			0.26
Methyl beta-D-galactopyranoside	C ₇ H ₁₄ O ₆	Monosaccharides			11.6			1.37
Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	saturated fatty acid			13.1			0.84
Neophytadiene	C ₂₀ H ₃₈	diterpene			13.9			0.30

2-Pentadecanone, 6,10,14-trimethyl	C ₁₈ H ₃₆ O	Diterpenoids			14.0			0.30
Oleic Acid	C ₁₈ H ₃₄ O ₂	Unsaturated fattyacids			15.1			0.42
Octadecanoic acid	C ₁₈ H ₃₆ O ₂	Unsaturated fattyacids			17.2			10.20
Heptadecanoic acid, ethyl ester	C ₁₉ H ₃₈ O ₂	Fatty esters			17.5			2.23
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C ₁₉ H ₃₈ O ₄	Fatty esters			20.3			17.74
9,12-Octadecadienoic acid (Z, Z) -, 2,3-dihydroxypropyl ester	C ₂₁ H ₃₈ O ₄	Glycerolipids			21.9			2.01
Octadecanoic acid, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₂ O ₄	Glycerolipids			22.2			11.95

2.7. Phytochemical analyses result of *E. elaterium* extracts

In the qualitative phytochemical content analysis, it was determined that the extracts contain glycosides and terpenoids but not saponins, phenols, and flavonoids. As seen in Table 2, it has been qualitatively determined that there are no phenolic, flavonoid structures in the ethanol extracts of the seed, fruit and juice parts of the *E. elaterium* plant, but only dense glycoside, terpenoid structures.

Table 2: Phytochemical contents of *E. elaterium* by qualitative analysis

<i>E. elaterium</i>	Saponin	Phenol-Tannin	Terpenoids	Flavonoids	Glycosides
Seed	----	----	----	----	----
Fruit	----	----	+	----	+
Juice	----	----	+	----	+

---: Absence + : Present

2.8. Antioxidant activity of *E. elaterium* extracts

We used DPPH free radical to determine the free radical scavenging activities of *E. elaterium* fruit, seed and juice ethanol extracts. The result showed an increase in free radical scavenging activity due to the increase in concentration. The free radical removal activity of the juice extract was the highest compared to the control (Figure 1). The % inhibition values of BHT, fruit, seed and juice extracts at the highest concentration (30 mg/ml) in free radical removal activity were calculated as 98.78%, 29.39%, 54.12%, and 89.45%, respectively. The concentration causing 50 % inhibition (IC₅₀) of the extracts was determined. Among the extracts studied, *fruit* (IC₅₀=53,48 mg/ml) had the lowest antiradical activity, while juice (IC₅₀=11,12 mg/ml), and seed (IC₅₀=25,54 mg/ml) were found to have the highest antiradical capacity (p<0.05). That agrees with the studies conducted by Gaballa et al. (2017), Felhi et al. (2017) and Bourebaba et al. (2020), who showed using DPPH assay that *E. elaterium* extracts have an antioxidant effect [16-18].

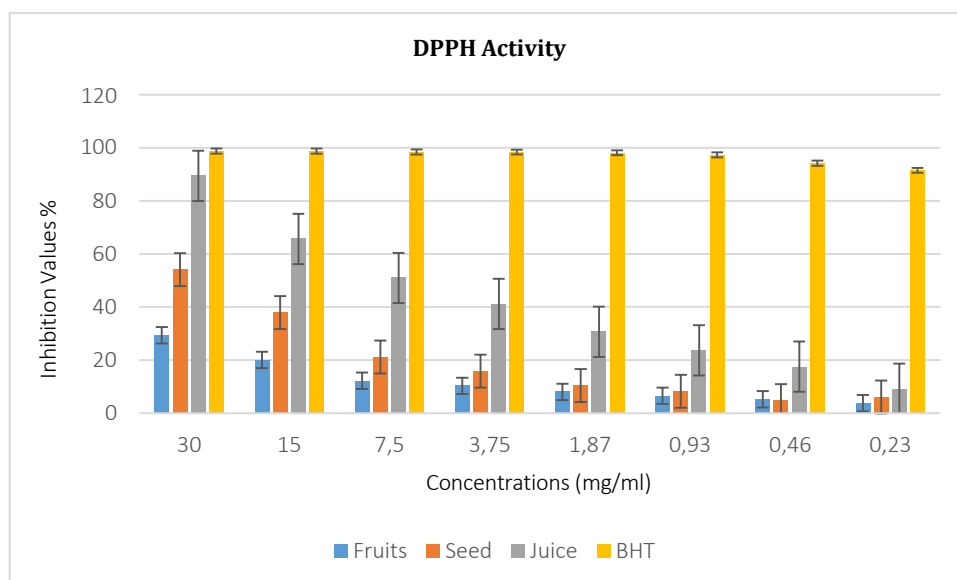


Figure 1. Free radical removal activity results of *E. elaterium* extracts

2.9. DNA interaction of *E. elaterium* extract

According to the results of DNA interaction studies examined by agarose gel electrophoresis method, *E. elaterium* fruit, seed and juice ethanol extracts were found to have a separating effect on plasmid DNA (Fig.2). All of the extracts affected on the uncoiling of the supercoiled plasmid DNA structure. There was a decrease in form I density and an increase in form II density. In addition, the formation of the linear DNA structure was determined by observing the Form III structure (Figure 2). Therefore, *E. elaterium* fruit, juice and seed ethanol extracts affect on DNA. These results agree with Abu-Hijleh et al. (2018) study that evaluated the genotoxic effects of *E. elaterium* fruit and leaf ethanol extracts [19]. These effects were determined using ERIC-PCR and SDS-PAGE. It showed a change in the DNA and protein profiles of *Escherichia coli* strains compared to control extracts. These changes were among the decreased or increased intensity of some bands, the absence or appearance of newly amplified fragments [20].

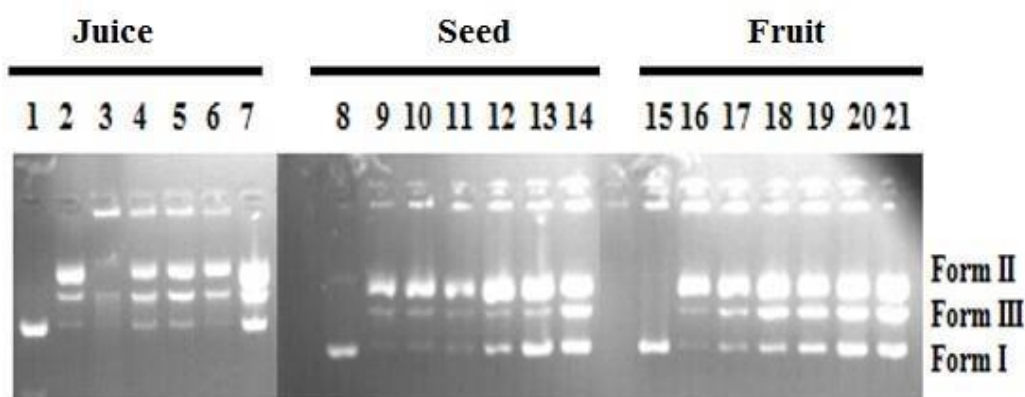


Figure 2. *E. elaterium* extracts-DNA interaction results: (1-8-15) 10% pBR322 DNA control, (2-9-16) 100 ppm, (3-10-17) 50 ppm, (4-11-18) 25 ppm, (5-12-19) 12.5 ppm, (6-13-20) 6.25 ppm, (7-14-21) 3.125 ppm.

2.10. Cytotoxic effect of *E. elaterium* extract

Cytotoxic effects of *E. elaterium* seed, fruit and juice extracts were determined using MTT assay. In the study HepG2 liver cancer line were used. The cytotoxic effect of ethanol extracts of *E. elaterium* plant parts on human liver cancer line (HepG2) is shown in Figure 3. Results determined that there is a decrease in the viability of HepG2 liver cancer cells due to increasing concentrations of *E. elaterium* ethanol extracts, especially seed extract has a greater effect on cell viability. As a result of the study, it was determined that juice, fruit and seed extracts caused a decrease in cell viability due to increasing concentrations. Cell viability of juice, fruit and seed extracts at the highest concentration of 1000 µg/ml was determined as 59.49%, 67.68% and 59.49%, respectively. The inhibitory effect of tumor cell proliferation was dose-dependent with a half-maximal inhibition (IC₅₀) of 309.02µg/ml, 525.43µg/ml and 549.54 µg/ml for juice, fruit and seed extracts respectively after 48-h incubation.

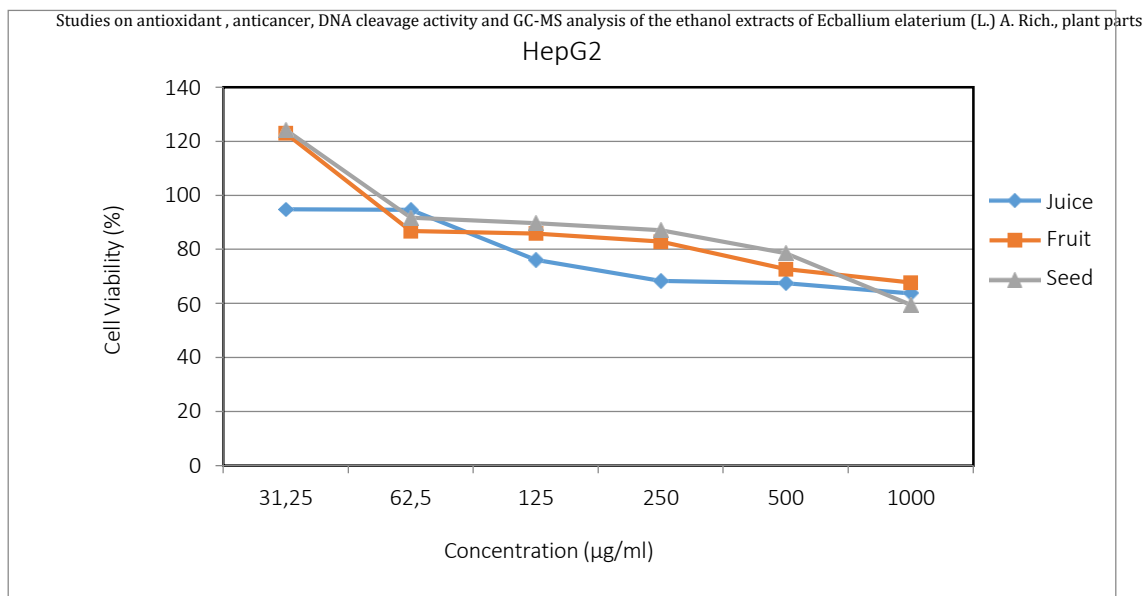


Figure 3. Cytotoxic activity of *E. elaterium* extracts on human liver cancer line (HepG2)

Ljubuncic et al. (2005) have previously reported the cytotoxic effect of an aqueous extract of *E. elaterium* on cell line with an IC_{50} of 1,000 µg/ml at 48-h incubation [20]. This concentration is close to IC_{50} value of the extract on the HepG2 cell line in our study (for fruit extract 525.43µg/ml at 48-h incubation).

Their results agree with Touihri et al (2019) study that determined that the seed oil had a strong antiproliferative effect on human colon adenocarcinoma (HT29) and fibrosarcoma (HT1080) cell lines [21]. The presence of glycoside (glucose ester, ether structures) detected in the extracts as a result of phytochemical analysis is compatible with the presence of cucurbitacin in the GC-MASS analysis. The decrease in cell viability in the HepG2 cell line, based on increasing concentration, indicates that the extract will show the anticancer activity of cucurbitacin B [22] (Berbard et al. 2010).

Similar results Karimi et al. (2016), Barakati et al. (2016), Bohlooli et al. (2012) and Hamidi et al. (2020) who tested *E. elaterium* extracts against gastric cell line (AGS), human brain cancer cell line (U87), AGS (human gastric carcinoma) and KYSE30 (human esophageal squamous cell carcinoma), (MCF-7, MDA-MB-468 and MKN-45) cancer cell lines respectively [23-26]. Their results agree with our results and demonstrate the cytotoxicity of *E. elaterium* against various cancer cell lines. In light of the data revealed by this study, it is necessary that the studies can be made more specific by purifying the cucurbitacin B containing glycoside structure with more specific, exact analyses to optimize the active ingredients and especially to observe the results of in vivo experiments in order to use *E. elaterium* against cancer.

3. Conclusion

This study provides an overview of the chemical composition and biological activity of juice, fruit and seed ethanol extract of *E. elaterium*. According to the results of the current study, cucurbitacin B, which is considered the most effective component in the *E. elaterium* plant, was detected in fruit peel and fruit juice extracts. It was determined that fruit juice, fruit and seed ethanol extract of *E. elaterium* showed antioxidant activity and nuclease activity on

DNA. It has also been determined that the extracts have a cytotoxic effect on liver cancer cell lines by reducing cell viability. According to the results, it is thought that fruit juice, fruit and seed ethanol extracts of *E. elaterium* can be used as anticarcinogenic agents.

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Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

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