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Investigation of Anticancer, Antioxidant, and Phytochemical Properties of *Cyperus esculentus* Extracts Obtained Using Different Solvents

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Abstract: The chemical constituents of extracts from *Cyperus esculantus* were analyzed by GC-MS. Sixteen compounds were identified in the chloroform extract, accounting for 93.75% of the total oil that consisted mainly of hexadecanoic acid, methyl ester (CAS) (46,47%), 2(3H)-furanone, dihydro-3-(phenylmethyl)-(CAS) (24,60%). Seventeen compounds were identified methanol and hexan extract, accounting for 93.75% of the total oil that consisted mainly of respectively cheilanth-13(14)-enic methyl ester (12,03%), 1,4-benzenediol,2,5-bis(1,1-dimethylethyl) (10,20%), cholesta-9 (11), 20 (22)-dieene-3,23-dione, 6-hydroxy-5. Alpha, 6.alpha) (13,35%) and chlorhhiophps sulfoxide (11,83%). The extracts showed significant activities against the two "classical" cell lines DU-145 and PC-3 with X-chellingence. Chloroform, methanol, and hexan extracts against the DU-145 cell line with the IC50 values of 1,73461 g/mL, 101,409 µg/mL, and 32,146 µg/mL, respectively. Chloroform, methanol, and hexan extracts against the PC-3 cell line with the IC50 values of 1.222, 1.941, and 2.081 µg/mL, respectively. The antioxidant activity of *Cyperus esculentus* extracts obtained using various solvents was evaluated through different in vitro antioxidant assays. The methanol extract of Cyperus esculentus exhibited higher activity compared to the other extracts. Among the extracts, the chloroform extract showed the strongest DPPH scavenging activity.

1. Introduction

Cyperus esculentus L. (Tiger nut) is a small, sweet tuber produced by the roots of an edible, perennial herbaceous plant. It is commonly found in Mediterranean countries (Coşkuner et al., 2002). The species *Cyperus esculentus* was first described by Linnaeus in 1771. Although subsequent studies evaluated the species at different taxonomic levels within the genera Pterocyperus, Pycreus, and Cyperus, the currently accepted classification of the species is C. esculentus (Şapcı, 2016). In addition, it grows in both tropical and temperate regions and can also be found in the wild; it is also referred to as chufa, habbulleziz, and yellow ground almond (Sidohounde et al., 2014; Bilgili et al., 2018; Anonymous, 2019a; Anonymous, 2019b; Yılmaz, 2019). Known by various names such as chufa, yellow nut sedge, earth almond, and ground almond, Cyperus esculentus is a perennial plant naturally distributed across tropical and temperate regions. It is widely cultivated in parts of Africa and China, as well as in several regions of Europe especially Spain, the Arabian Peninsula, and many areas of Asia (Şapcı, 2016; Abdelkader et al., 2017). The tubers of this plant are commonly used in confectionery production (Zohary, 1986). Cyperus esculentus has an erect, triangular stem, yellowish-green leaves, and a height ranging from 20 to 60 cm. Its rhizomes store protein, starch, and other nutrients that contribute to the formation of its tubers. Its flowers range in color from golden yellow to brown (Abdelkader et al., 2017). Due to its rapid growth, it is often mistaken for a weed in some areas. It grows best in well-drained sandy or loamy soils with a pH between 5.0 and 7.5 and thrives at temperatures above 20°C (Dyer, 2009). Thanks to the valuable compounds found in its tubers, Cyperus esculentus has economic importance in various sectors, including the food industry, animal feed, paper and fiber production, the pharmaceutical industry, and as an ornamental plant (Şapcı, 2016). In some parts of the world, Cyperus esculentus (Tiger Hazel) is widely used as a healthy food for both humans and animals due to its many nutritional and functional properties. The underground tuber of the tiger nut is called chufa. It can be consumed fresh, soaked, roasted, or dried. These tubers are rich in energy (~400–450 kcal/100 g) and contain approximately 26–30% and 21–25% starch and fat, respectively. They also contain protein (3–8%), fiber (8–10%), various vitamins (E and C), and minerals such as phosphorus and potassium (Bosch et al., 2005; Arafat et al., 2009; Chinma et al., 2009; Chukwuma et al., 2010; Adejuyitan, 2011; Tigernuts Traders, 2014). The main fatty acids in tiger nut oil are monounsaturated fats (>60%), making its profile comparable to olive oil or hazelnut oil (Dubois et al., 2007). Recent research and studies on this plant have primarily focused on its organoleptic properties, phytochemical composition, oil content, biochemical activities, and nutritional value. However, its medicinal properties are rarely discussed, despite its well-known traditional medicinal use in some regions (Achoribo et al., 2017). Tiger hazel has been used as a health herb for over 4000 years due to its mineral, energy, and oleic acid content. It also contains high amounts of arginine (Bamishaiye and Bamishaiye, 2011). Thanks to its phosphorus, potassium, calcium, magnesium, and iron content, tiger hazel is beneficial for bones, tissue repair, muscles, blood circulation, and supports body development (Mohdaly, 2019). Moreover, tiger nut has been reported to have protective effects against cardiovascular diseases (Borges et al., 2008; Chukwuma et al., 2010; Tigernuts Traders, 2014), and due to its vitamin E content, it plays a role in red blood cell formation and function, and is also effective against cancer (Gambo and Da'u, 2014). The vitamin C present in tiger nut is a good antioxidant, promotes iron absorption, helps maintain vitamin E levels, and provides essential protection for the immune system and tissues (Roselló-Soto et al., 2019). In Turkey, the cultivation of tiger nut is very limited and is carried out in small quantities at the Eastern Mediterranean Agricultural Research Institute in Adana. While in Spain, the tubers are consumed as human food, they are also reported to be used in the pharmaceutical and perfumery industries, as well as in fish feed, turkey, and chicken feed production (Nazlıcan, 2007).

2. Material and Method

2.1. Sample extraction

All above-ground parts of the collected plant samples were dried in a sunless environment and then crushed using a food processor. 13-17 grams of this powdered sample were weighed and dissolved in 200 mL of organic solvent. Chloroform, hexane, and methanol were used as solvents. After extraction for 6 hours using a 250 ml Soxhlet apparatus, the solvents were separated using an evaporation device, and the resulting extracts were stored at +4°C (Doğaner et. al., 2014).

2.2. GS-MS

In this study, extracts were prepared from *Cyperus esculentus* (ground almond) tubers using chloroform, methanol, and hexane solvents, and the resulting extracts were then analyzed using a GC-MS (SHIMADZU, QP2010 ULTRA) instrument. The aim was to comparatively determine the chemical components obtained according to the type of solvent. As a result of the analysis, various phytochemicals specific to each extract were identified (Kilani et al., 2008).

Sample: Cyperus esculentus tubers

Solvents: Chloroform, methanol, hexane

Extraction: A separate cold extraction method was applied to each solvent.

GC-MS instrument: (Instrument model can be added here if available)

Column type: DB-5MS capillary column

Carrier gas: Helium

Injection temperature: 250 °C

Oven program: Gradual heating between 60–300 °C

Spectral library: NIST/Wiley databases

2.3. DPPH radical scavenging capacity

In the analysis of DPPH radical scavenging capacity, 1.5138 g of Tris (Trizma base, MW: 121.1 g/mol) was dissolved in 200 ml of deionized water, HCl was used to adjust the pH to 7.4, and the volume was completed to 250 ml with distilled water. Thus, Tris-HCl buffer (50 mM, pH 7.4) was prepared. The DPPH solution was prepared by dissolving 9.86 mg DPPH in 250 mL of methanol and stored in a dark bottle.

The DPPH radical scavenging activity of *Cyperus esculentus* extracts was determined according to the method of Brand-Williams et al. (1995). Different concentrations were tested for each antioxidant. Results were expressed as the molar ratio of the antioxidant to DPPH. For the experiment, 450 µl of Tris-HCl buffer (50 mM, pH 7.4), 1 ml of 0.1 mM DPPH methanol solution, and 50 µl of extract were mixed. A reagent mixture without extract was used as a control, and catechin was used as a positive control. The mixtures were incubated in the dark at room temperature for 0, 1, 15, and 30 minutes. The measured absorbance value was 515 nm.

The DPPH concentration in the reaction medium (CDPPH) was calculated using the following calibration equation:

$$DPPH_{515}nm = 3.6852 \times (CDPPH) + 4.9334$$

Antioxidant activity was defined as the amount of antioxidant required to reduce DPPH concentration by 50% (IC50). The IC50 value represents the antioxidant concentration that

causes a 50% reduction in the initial DPPH concentration. The time required to reach equilibrium concentration was calculated from kinetic curves and defined as TEC50 (Sánchez-Moreno et al., 1998). All analyses were performed in triplicate, and mean values were used.

2.4. Determination of total phenolic content

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method described by Sağdıç and Özcan (2002). For the experiment, 40 µL of extract was added to test tubes, followed by 2.4 mL of distilled water. Then, 200 µL of Folin-Ciocalteu reagent, 600 µL of sodium carbonate solution, and 760 µL of distilled water were added to the mixture and stirred for 15 seconds. Reduction of the Folin-Ciocalteu reagent in an alkaline environment resulted in the formation of a blue color. It was left to stand at room temperature for 2 hours. Subsequently, absorbance was measured at 765 nm using a spectrophotometer. All measurements were performed in triplicate, and the results were expressed as gallic acid equivalents per liter (GAE).

2.5. Cytotoxic activity

2.5.1. Cell lines used in the study

The cell lines used in this study were obtained from the cell bank of the Department of Biochemistry, Faculty of Pharmacy, Erciyes University.

2.5.2. Cell culture

Cells were cultured at 37°C in a sterile incubator containing 5% carbon dioxide. The cells were maintained in RPMI medium supplemented with 10% fetal bovine serum (FBS) that had been heat-inactivated at 55°C for 30 minutes, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine. Cells were cultured in 75 cm² culture flasks and transferred to new flasks using 0.25% trypsin/EDTA to separate them from the surface (Freshney, R. I. (2016), Riss, T. L. et al. (2019)). The cell lines used in our study were obtained from the Erciyes University Drug Application and Research Center cell bank.

2.6. Real-time cell analysis (xCELLigence)

The xCELLigence system was used to monitor the real-time effects of C. esculentus extracts on DU-145 and PC-3 cells according to the manufacturer's instructions (Ke et al. 2011). The xCELLigence system was used to monitor the real-time effects of C. esculentus extracts on DU-145 and PC-3 cell lines according to the manufacturer's protocol (Ke et al., 2011). The system consists of four main components: an RTCA analyzer, an RTCA SP station, an RTCA computer with integrated software, and a disposable E-plate 96 (Figure 1). The RTCA SP station was placed inside the incubator, while the analyzer and the laptop equipped with software were positioned outside.

E-plate 96 is a disposable, single-use platform designed for cell-based experiments using an RTCA SP system. Unlike conventional 96-well microtiter plates, E-plate 96 incorporates integrated gold microelectrode sensor arrays at the bottom of each well, enabling the detection of impedance changes caused by cells within the wells (Bird et al., 2009; Ke et al., 2011). Impedance changes can be recorded at intervals as short as two minutes, allowing for continuous monitoring of cellular physiological responses.

The impedance detected between the electrodes in each well is affected by the electrode configuration, the number of cells, and the degree of cell adhesion to the electrode surface. When cells adhere to the electrode sensors, they alter the local ionic environment at the electrode-solution interface, causing an increase in impedance. The cell index reflects cell adhesion, morphological changes, and cell separation associated with cell death. Therefore, the cytotoxic effects of the tested substances can be assessed through changes in the cell index (Ke et al., 2011).

DU-145 and PC-3 cells were cultured in flasks, and when they reached approximately 80% density, the cells were separated and seeded onto the E-plate 24. After seeding, the plates were incubated at room temperature for 30 minutes to allow the cells to settle. The E-plates were then transferred to 96 incubators, and different cell densities (50,000, 25,000, 12,500, 6,250, and 3,125 cells per well) were tested to determine the optimum seeding concentration. Based on the achievement of a logarithmic growth phase, the optimum seeding densities were determined to be 20 × 10^3 cells/well for DU-145 cells and 10 × 10^3 cells/well for PC-3 cells. Cell attachment, proliferation, and dispersal were monitored every 15 minutes by measuring impedance changes in the E-plate wells.

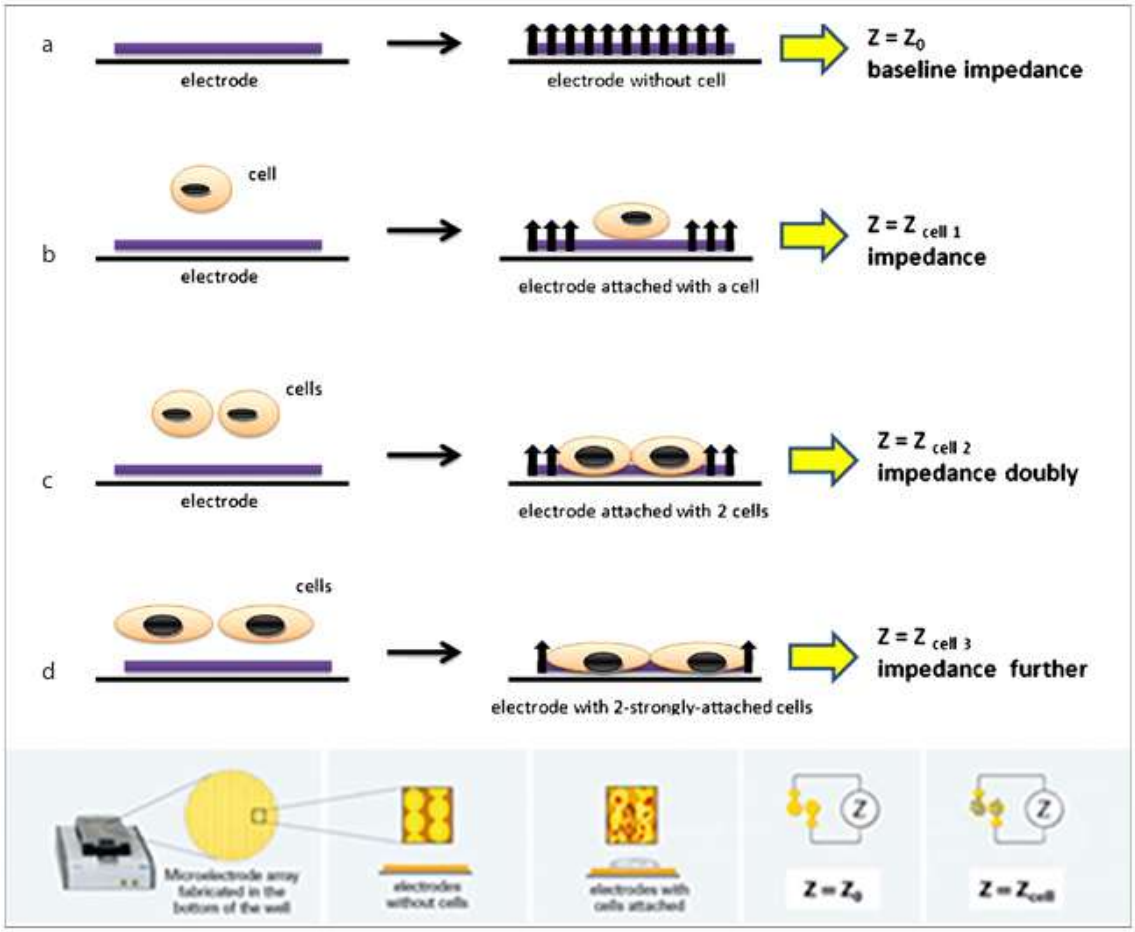


Figure 1: The bottom of the 96-well E-plate contains gold electrodes that are responsive to cell attachment, growth, and size increase. In the absence of cells (a) on the well bottom, the measured impedance is zero ($Z = Z_0$). The presence of adherent cells modifies the impedance (b), while an increased number of cells (c) leads to a further rise in impedance. Cell enlargement also influences impedance (d); when cells increase in size without undergoing mitotic division, impedance changes accordingly, and the cell index increases (Dokumacı et al., 2019).

2.6.1. Cytotoxicity assay using Xcelligence system

To determine the cytotoxic effect of hexane, chloroform, and methanol on DU-145 and PC-3 cell lines, RTCA (xcelligence) was used. A total of DU-145 10*103 cells/ well and PC-3 10*103 cells were seeded in the E-plate 96 wells, and approximately 24h post-seeding, when the cells were in the log growth phase, the cells were treated with extracts and only medium control. Controls received medium and treated groups (all extraction at 400, 200, 100, 50, 25, 12,5 µg/mL concentrations) were replicated 4 times, and the experiments were run for 96 h. All calculations were done with the RTCA-integrated software of the xCelligence system. The

RTCA software performs the curve-fitting of selected "sigmoidal dose-response equation" and calculates the logarithmic half maximum effect of concentration [log (IC50)] values at a given time point based on log concentration producing 50% reduction of cell index (CI) value relative to the control CI value (100%). Cell index value represents the alteration of cell viability, cell attachment, and cellular growth rate. Likewise, when the cell index data is obtained during the experiment at the particular time points, dynamic monitoring of the cells' response can be elucidated.

2.7. Statistical analysis

For each experimental group, results were obtained from a minimum of three independent experiments. All statistical evaluations were performed using one-way analyses of variance (ANOVA), followed by Bonferroni post hoc tests. The same statistical approach was applied to calculate the standard deviation (SD). Differences between the groups were considered statistically significant at $p < 0.05$, as determined using the same software. Data are expressed as mean ± SD.

3. Results

3.1.GS-MS

3.1.1. Methanol extract (chromatogram I)

The total ion chromatogram (TIC) of the methanolic extract of *Cyperus esculentus* tubers, recorded over a total run time of approximately 104 min, exhibits a highly complex and information-rich chemical profile. The chromatogram is characterized by numerous well-resolved peaks with good signal-to-noise ratios, indicating efficient chromatographic separation and the presence of a wide range of polar and semi-polar phytochemicals.

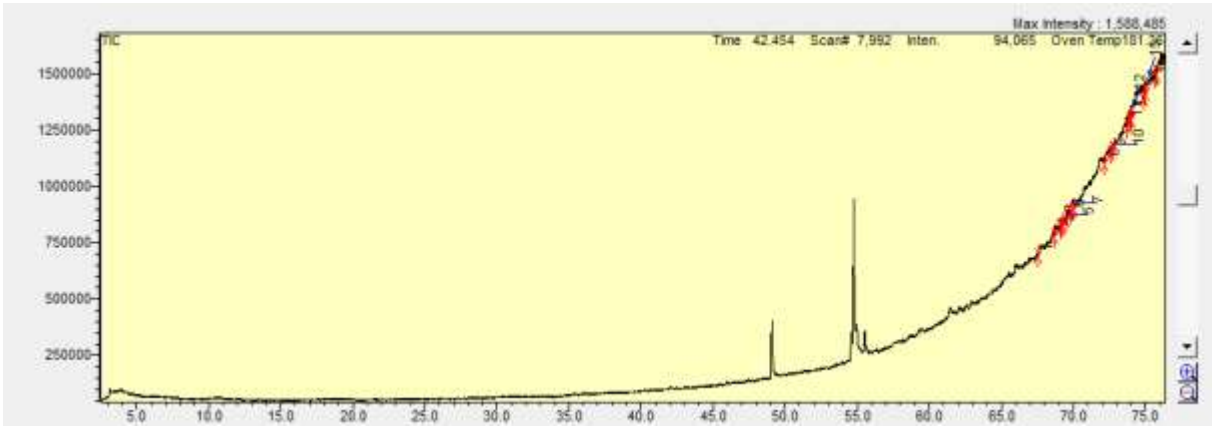
The early retention time region is dominated by low-molecular-weight oxygenated compounds, including 2-furanmethanol, 1,2-cyclopentanedione, 4-oxo-5-methoxy-2-penten-5-olide, and 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one. These compounds are typically associated with carbohydrate degradation products, Maillard-type reaction intermediates, and lactone derivatives. Such constituents are well known for their antioxidant and redox-active properties, suggesting a strong contribution of these early-eluting compounds to the pronounced antioxidant activity observed for the methanol extract.

This region represents the most intense section of the chromatogram, with several dominant peaks corresponding to oxygenated monoterpenes and cyclic ketones, such as trans-α-carveol, D-limonene-2-propyl, p-cymen-7-ol, and 2-cyclohexanedione derivatives. The abundance of these terpenoid-related compounds highlights the ability of methanol to extract moderately polar secondary metabolites. These compounds have been widely reported to exhibit antioxidant, antimicrobial, and cytotoxic activities, which is consistent with the significant biological effects recorded for this extract.

A notable peak assigned to 2-furanone, dihydro-3-(phenylmethyl)- further emphasizes the prevalence of lactone-type structures, which are often implicated in radical scavenging mechanisms and apoptosis-related cytotoxicity.

In the late retention region, fatty acids and fatty acid methyl esters such as hexadecanoic acid methyl ester, n-hexadecanoic acid (palmitic acid), 9,12-octadecadienoic acid methyl ester, and 8,11,14-docosatrienoic acid methyl ester become predominant. These compounds contribute substantially to the total chromatographic area and reflect partial co-extraction of lipophilic constituents despite the polar nature of methanol.

The final segment of the chromatogram reveals the presence of high-molecular-weight sterols and triterpenoid derivatives, including cholesta-5,6-dien-3-ol benzoate (β-sitosterol derivative) and campesterol.



Peak#	Ret.Time	Start Tm	End Tm	m/z	Area	Area%	Height	Height%	A/H	Mark	Name
1	67.605	67.540	67.825	TIC	82208	4.69	23355	3.89	3.52		d-Ribitol, 1,4-anhydro-1-C-(2,6-diamino-3-pyridinyl)-2,3-O-(1-methylethylidene)-, (1S)-
2	68.712	68.660	68.750	TIC	211075	12.03	46090	7.67	4.58	V	Cholanth-13(14)-enic Methyl Ester
3	69.117	69.095	69.135	TIC	42535	2.45	21043	3.50	2.04	V	30-Norlupan-28-oic acid, 3-hydroxy-21-methoxy-20-oxo-, methyl ester, (3.beta.)-
4	69.230	69.210	69.265	TIC	41449	2.36	17687	2.95	2.34	V	14.alpha.-Cholanth-12-enic Methyl Ester
5	69.495	69.455	69.510	TIC	41348	2.36	25948	4.32	1.59	V	d-Xylitol, pentaacetate (CAS)
6	69.836	69.805	69.845	TIC	58142	3.31	26561	4.42	2.19	V	lupeol,lupan-3-ol
7	70.035	70.010	70.055	TIC	81052	3.48	27927	4.65	2.19	V	Bremporidol
8	72.160	72.120	72.190	TIC	106030	6.09	29963	4.99	3.57	V	Cholanth-13(14)-enic Methyl Ester
9	72.634	72.615	72.660	TIC	67273	3.84	32218	5.36	2.09	V	METHYL 7-ETHYL-10-HYDROXY-11-HYDROXY(ISO)-3,11-DIMETHYL-2,6-TRIDECADIENOATE
10	72.797	72.765	72.835	TIC	51287	2.92	24136	4.02	2.13	V	2,6-Lutidine 3,5-dichloro-4-dodecylthio-
11	73.779	73.705	73.796	TIC	178594	10.20	43200	7.19	4.14	V	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-
12	73.960	73.935	73.985	TIC	162123	9.24	56319	9.38	2.88	V	beta.-n-Butylether of 11-epi-dihydroxeritrinin
13	74.015	73.985	74.055	TIC	233950	13.35	59909	9.98	3.91	V	Cholesta-9(11),25(27)-diene-3,23-dione, 6-hydroxy-, (3.alpha.,6.alpha.)-
14	74.853	74.840	74.886	TIC	125256	7.14	62096	10.35	2.02	V	14.alpha.-Cholanth-12-enic Methyl Ester
15	75.038	75.000	75.060	TIC	163078	9.34	51963	8.65	3.15	V	1,4-Benzenedicarboxylic acid, bis(4-octylphenyl) ester
16	75.684	75.650	75.706	TIC	41308	2.35	20842	3.47	1.98	V	2-Pyrrolyroyl-glycinamide
17	75.794	75.730	75.810	TIC	85024	4.85	31306	5.21	2.72	V	Ginsenol

Figure 2: Methanol extract chromatography results and contents

3.1.2. Hexane extract (chromatogram II)

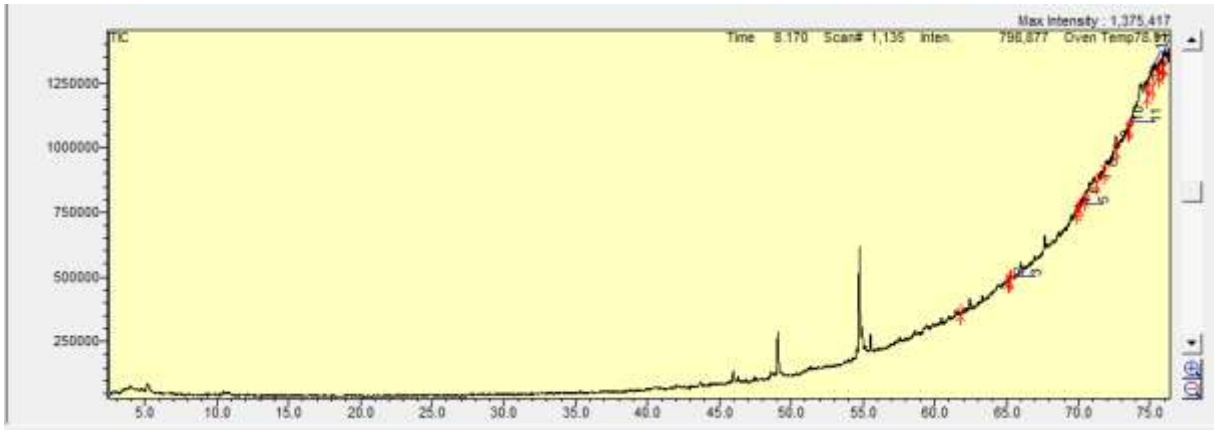
The TIC of the hexane extract shows a markedly different chemical profile compared to the methanol extract, reflecting the strong influence of solvent polarity on extraction selectivity. The chromatogram is dominated by fewer but more intense peaks, primarily corresponding to non-polar and lipophilic constituents.

Only a limited number of early-eluting compounds are observed in this region, indicating minimal extraction of low-molecular-weight polar constituents. Minor peaks correspond to small hydrocarbons and weakly oxygenated terpenes, present at relatively low intensities.

The mid retention region contains a moderate number of peaks associated mainly with non-polar terpenoid hydrocarbons and simple aromatic derivatives. Compared to the methanol extract, oxygenated monoterpenes are less abundant, consistent with the low polarity of hexane.

This region dominates the hexane TIC and is characterized by very intense peaks corresponding to long-chain fatty acids and fatty acid methyl esters, including hexadecanoic acid methyl ester, 9,12-octadecadienoic acid methyl ester, and related unsaturated fatty acid derivatives. These compounds account for a significant proportion of the total chromatographic area and explain the pronounced lipophilic nature of the extract.

Sterol-like compounds and triterpenoid derivatives, such as cholestane-related structures, are detected in this region. Their presence, although at lower relative abundances, is notable due to their known membrane-modulating and antiproliferative effects.



Peak#	Ret.Time	Start Tm	End Tm	m/z	Area	Area%	Height	Height%	A/H	Mark	Name
1	61.806	61.770	61.870	TIC	40446	3.12	12233	1.69	3.96	V	Budol
2	65.216	65.170	65.250	TIC	95249	6.14	24908	3.85	3.82	V	a-Xylitol, pentaacetate (CAS)
3	65.269	65.250	65.300	TIC	44111	2.84	17543	2.71	2.51	V	4a,beta.-methyl-1,1a,beta.,2,3,4,4a-hexahydro-5(8H)-cycloprop[d]n
4	69.980	69.930	69.970	TIC	63717	4.11	29047	4.54	2.17	V	Cheilanth-13(14)-enic Methyl Ester
5	70.095	70.085	70.120	TIC	42637	2.75	23047	3.56	1.85	V	5,6,7-Trimethoxy-1-indanone
6	70.505	70.485	70.520	TIC	50362	3.25	26943	4.17	1.87	V	Ginsenol
7	71.296	71.270	71.315	TIC	42761	2.76	26228	3.13	2.11	V	Prednisolone Acetate
8	71.829	71.765	71.835	TIC	67279	4.34	23309	3.60	2.89	V	Haloxazolam
9	72.642	72.630	72.685	TIC	133748	8.62	57259	8.85	2.34	V	D-Mannitol, hexaacetate (CAS)
10	73.537	73.465	73.555	TIC	145286	9.36	37688	5.83	3.85	V	Cyclobarbital
11	73.618	73.555	73.630	TIC	97884	6.30	26894	4.16	3.64	V	14.alpha.-Cheilanth-13(14)-enic Methyl Ester
12	74.827	74.780	74.835	TIC	183446	11.83	64359	9.95	2.85	V	Chlorthiophos sulfoxide
13	75.148	75.115	75.150	TIC	138886	8.81	77308	11.94	1.77	V	Bromacil
14	75.605	75.575	75.615	TIC	86564	5.58	42357	6.55	2.04	V	Glutaric acid, di(2-isopropoxyphenyl) ester
15	75.631	75.615	75.650	TIC	94388	6.08	54636	8.45	1.73	V	Cheilanth-13(14)-enic Methyl Ester
16	75.918	75.870	75.920	TIC	125435	8.09	51862	8.02	2.42	V	Perhydro-ftx-2-one, 2-depentyl-, acetate ester
17	75.936	75.920	75.960	TIC	93432	6.02	56901	8.80	1.64	V	Tartronic acid, 4-(dimethylethylallyl)phenyl-, dimethyl ester

Figure 3: Hexane extract chromatography results and contents

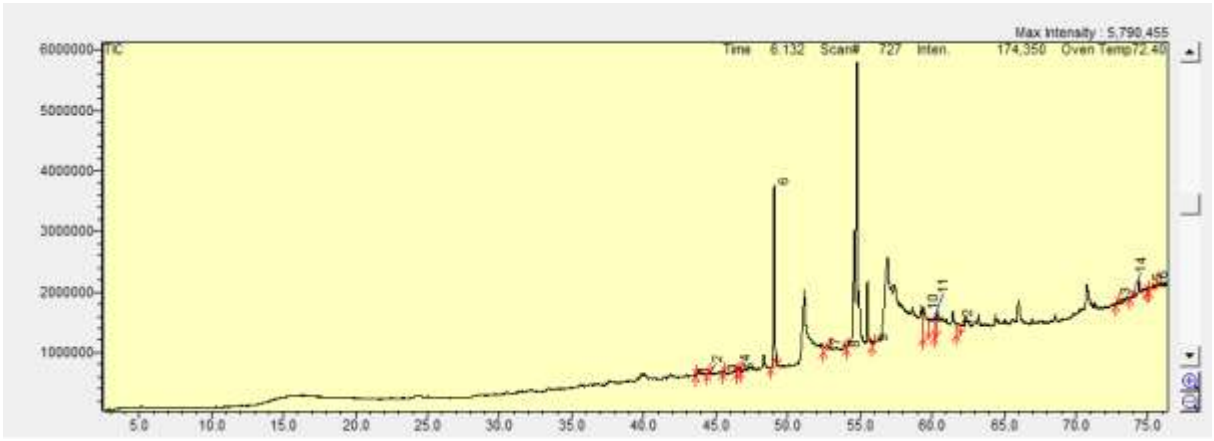
3.1.3. Chloroform extract (chromatogram III)

The TIC of the chloroform extract exhibits an intermediate chemical complexity between the methanol and hexane extracts, reflecting the semi-polar nature of chloroform.

The early region is dominated by low-molecular-weight oxygenated compounds such as 2,3-butanediol, 5-methylfurfural, 5,5-dimethyl-3-hepten-2-one, and 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one. Notably, 5-hydroxymethylfurfural (HMF) appears as a major component, occupying a substantial proportion of the total chromatographic area. HMF is widely recognized for its antioxidant and redox-active properties.

This region contains aromatic and furan-based compounds such as benzeneacetic acid and 1-(2-furanyl)-2-hydroxyethanone. The dominance of these oxygenated structures indicates that chloroform effectively extracts semi-polar compounds with strong biological relevance.

Only a limited number of late-eluting compounds are detected, including minor amounts of polycyclic aromatic hydrocarbons such as substituted naphthalene derivatives. The near absence of long-chain fatty acids and sterols beyond this region distinguishes the chloroform extract from the hexane extract.



Peak#	Ret.Time	Start Tm	End Tm	m/z	Area	Area%	Height	Height%	A/H	Mark	Name
1	43.647	43.635	43.705	TIC	69991	0.17	28915	0.60	2.60	V	Benzaldehyde methylimine
2	44.486	44.375	44.570	TIC	232884	0.56	30558	0.68	7.62		ISOCHIAPIN B %2=
3	45.551	45.486	45.655	TIC	180993	0.43	30348	0.68	5.96	V	Dimethyl 4-methyloctane-1,8-dioate
4	46.460	46.425	46.490	TIC	66422	0.16	20100	0.45	3.30	V	RORIDIN E
5	46.745	46.675	46.805	TIC	73751	0.18	15033	0.33	4.92	V	QUERCETIN 7,3',4'-TRIMETHOXY
6	48.058	48.875	49.290	TIC	19383633	46.47	3826435	67.29	6.41		Hexadecanoic acid, methyl ester (CAS)
7	52.716	52.505	52.875	TIC	1089168	2.57	70868	1.58	15.09	V	1,3,2-Dioxaborinane, 2-ethyl-4-methyl-
8	54.096	54.035	54.145	TIC	167622	0.40	32245	0.72	5.20	V	2,11-Dodecanediene (CAS)
9	55.971	55.825	56.035	TIC	463578	1.16	61388	1.37	7.66	V	Benzene, (1-methyl-1-propylpentyl)-
10	59.436	59.365	59.615	TIC	10250885	24.60	505406	11.26	20.28	V	2(3H)-Furanone, dihydro-3-(phenylmethyl)- (CAS)
11	60.190	60.140	60.315	TIC	3195513	7.67	306348	6.82	10.43	V	(Z)-3,7,11-Trimethyldodec-2-enoic acid, methyl ester
12	61.865	61.665	61.990	TIC	3209319	7.69	169161	3.77	18.96	V	ISOLONGIFOL-8-OL
13	72.861	72.740	72.990	TIC	276328	0.66	28473	0.63	9.70	V	Cheilanth-13(14)-enic Methyl Ester
14	73.940	73.740	74.065	TIC	628524	1.51	48441	1.08	12.96	V	14.alpha.-Cheilanth-13(14)-enic Methyl Ester
15	75.015	74.840	75.065	TIC	752369	1.81	63473	1.41	11.85	V	Benzothiophene-3-carboxylic acid, 4,5,6,7-tetrahydro-2-amino-6-eth
16	75.437	75.065	75.585	TIC	1651263	3.96	59713	1.33	27.65	V	Ginsenol

Figure 4: Chloroform extract chromatography results and contents

3.2. DPPH radical scavenging capacity

The DPPH scavenging activity was depicted in Figures 2,3,4. The chloroform, hexane, and methanol extracts all exhibit DPPH activity. Chloroform, hexane, and methanol extracts all exhibit DPPH activity. Concentrations are 0.1, 0.5, 1, 2, 4, and 5 mg/ml. In the chloroform extract, the observed values were 0.838+0.001, 0.488+0.002, 0.530+0.004, 0.310+0.002, 0.179+0.002, and 0.169+0.001, respectively. In the hexane extract, the values are 0.816+0.001, 0.586+0.001, 0.39+0.001, 0.236+0.001, 0.141, and 0.156+0.006, respectively. In the methanol extract, the values are 0.841+0.001, 0.546+0.001, 0.163+0.002, 0.099+0.105, and 0.110 mg/ml (Figure 5,6,7).

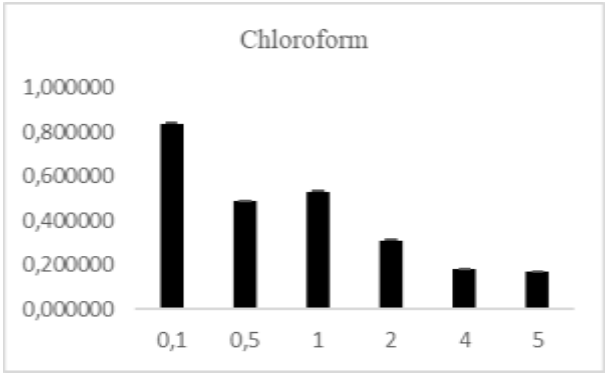


Figure 5: DPPH scavenging activity of the aqueous and chloroform extracts of *C. esculentus*

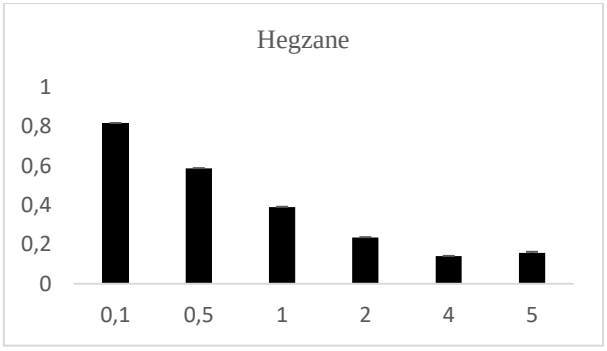


Figure 6: DPPH scavenging activity of the aqueous and Hexane extracts of *C. esculentus*

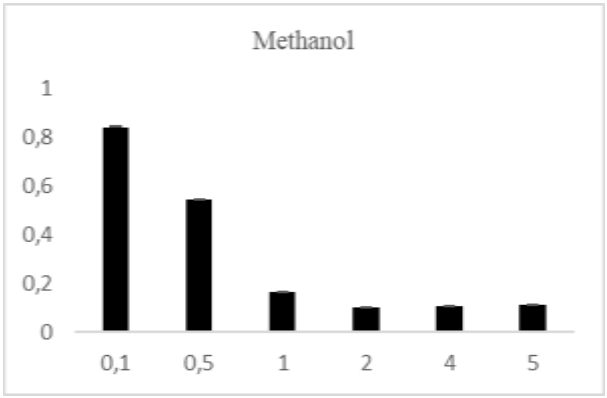


Figure 7: DPPH scavenging activity of the aqueous and methanol extracts of *C. esculentus*

3.3. Determination of total phenolic content

Figures 8, 9, and 10 depict the total phenol contents of both chloroform, hexane, and methanol aqueous extracts. In Figure 7, all extracts have shown a level of phenol content, with the highest value. All solvents had an affinity for phenolic compounds. As the concentration increased, the amount of phenolic compounds also increased. Total flavonoids are present in all solvents of the *C. esculentus* plant.

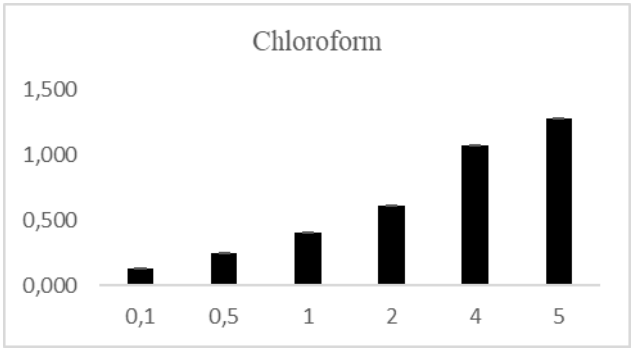


Figure 8: Total flavonoid content of the aqueous and chloroform extracts of *C. esculentus*

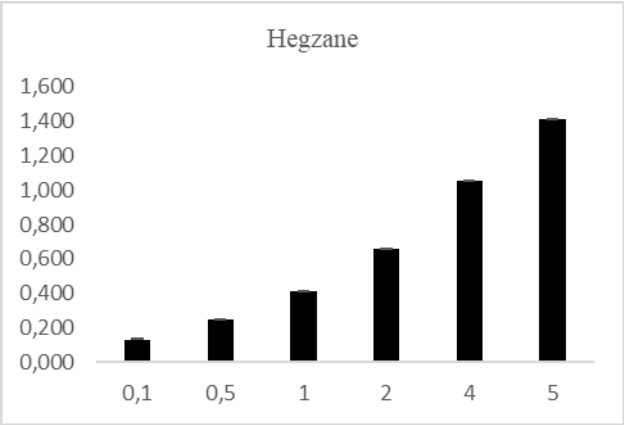


Figure 9: Total flavonoid content of the aqueous and hexane extracts of *C. esculentus*

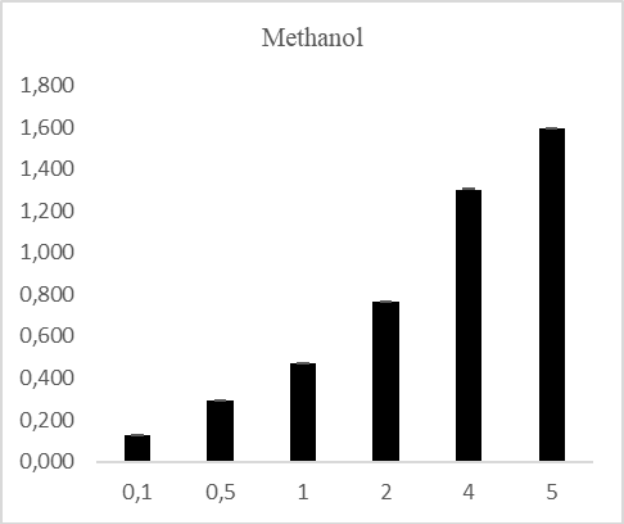


Figure 10: Total flavonoid content of the aqueous and methanol extracts of *C. esculentus*

3.4. Cytotoxic activity

3.4.1. Cytotoxic activity in DU-145 cell line

The effect of plant extracts obtained from different solvents on the viability of DU-145 cells was analyzed using an xCELLigence instrument after 24 hours of incubation with different doses (12.5-25-50-100-200-400 µg/mL) and control groups. The percentage cell viability graph obtained as a result of the analysis is given in Figures 11,12,13.

In cell DU-145, IC50 values were calculated for plant samples in different solvents (hexane, chloroform, and methanol) as 32.146 µg/mL, 1.73461 µg/mL, and 101.409 µg/mL, respectively. Accordingly, the most effective result was obtained with the hexane extract. Based on the results of extracts obtained from different solvents of *Cyperus esculentus L.*, the chloroform extract was observed to be ineffective.

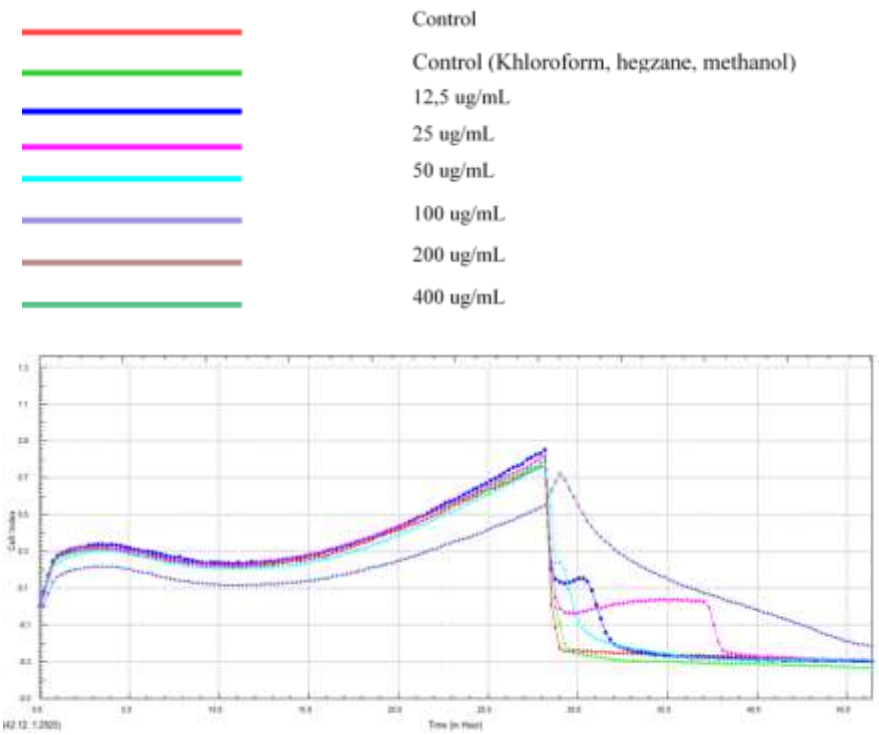


Figure 11: Cell viability in chloroform extract

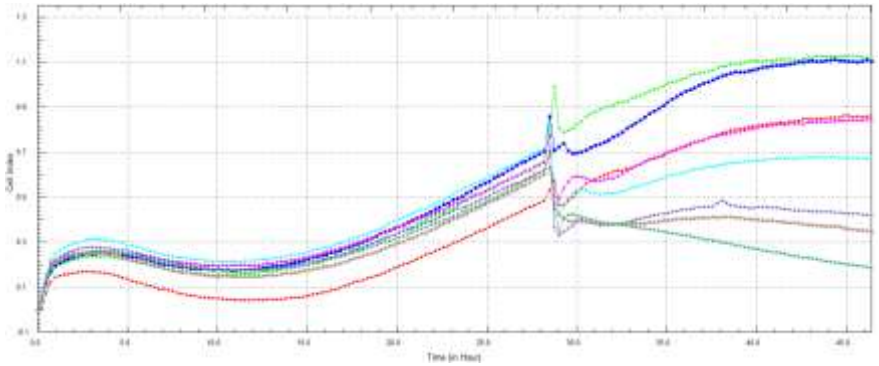


Figure 12: Cell viability in hexane extract

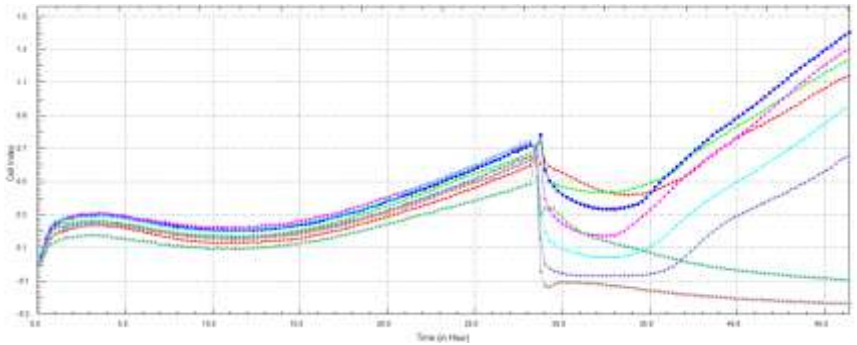


Figure 13: Cell viability in methanol extract

3.4.2. Cytotoxic activity in PC-3 cell line

The effect of plant extracts obtained from different solvents on PC-3 cell viability was analyzed using an xCELLigence instrument after 24 hours of incubation with different doses

(12.5-25-50-100-200-400 µg/mL) and control groups. The percentage cell viability graph obtained as a result of the analysis is given in Figures 14,15,16.

In the PC-3 cell, IC50 values were calculated for plant samples in different solvents (hexane, chloroform, and methanol) as 2.081 µg/mL, 1.222 µg/mL, and 1.941 µg/mL, respectively. Accordingly, the most effective result was obtained with the chloroform extract. Based on the results of extracts obtained from different solvents of *Cyperus esculentus L.*, it was observed that all extracts were effective.

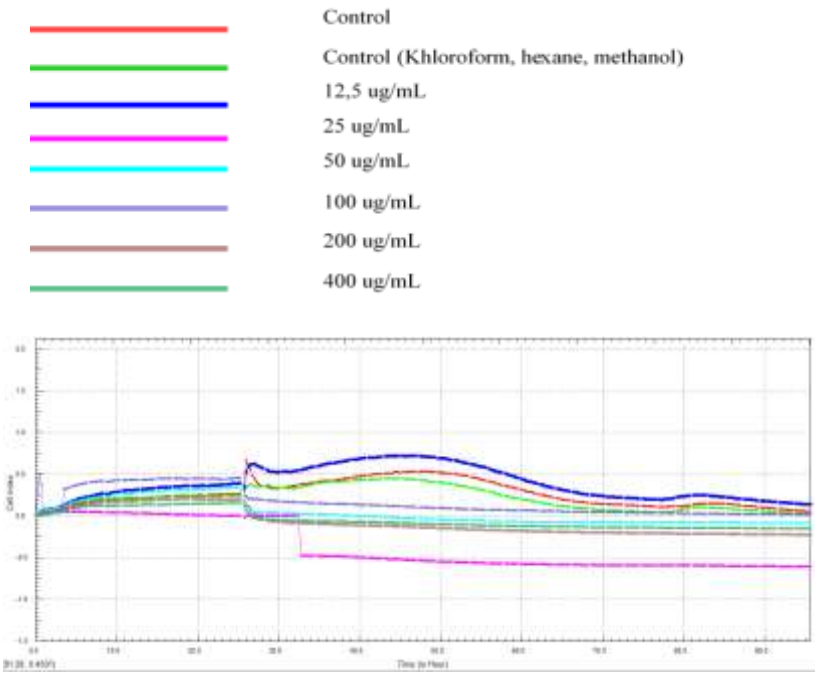


Figure 14: Cell viability in chloroform extract

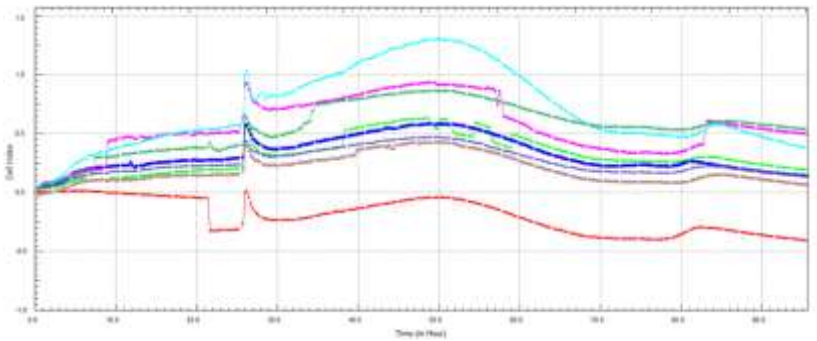


Figure 15: Cell viability in hegzane extract

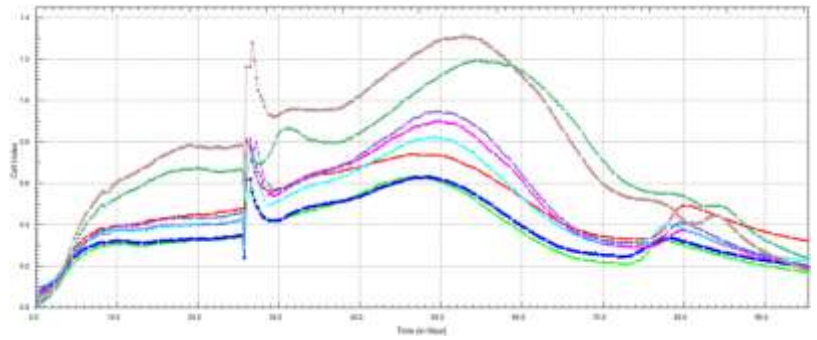


Figure 16: Cell viability in methanol extract

The results of the extracts in all cells are shown in the table below.

Table 1. Ekstrelerin DU-145 ve PC-3 hücrelerindeki inhibisyon aktiviteleri (ug/mL)

	Chloroform	**Hegzane**	**Methanol**
DU-145	32,146	1,73461 (g/mL)	101,409
PC-3	2,081	1,222	1,941

4. Conclusion

In this study, we evaluated the biological activities of extracts obtained from Cyperus esculentus (tiger nut) using different solvents on two prostate cancer cell lines: PC-3 and DU-145. The findings showed that the anticancer activity varied significantly, depending on both the type of solvent used and the specific characteristics of each cell line. The most notable inhibitory effect was observed in the PC-3 cell line when treated with the hexane extract, which had an IC_{50} value of 1,222 µg/mL. In contrast, the highest activity in the DU-145 cell line was detected with the chloroform extract. Notably, the hexane extract demonstrated no anticancer activity against DU-145 cells. These results indicate that the polarity of the solvent plays a crucial role in extracting biologically active compounds and in producing cell line-specific effects. Overall, Cyperus esculentus shows significant anticancer potential against PC-3 prostate cancer cells. Therefore, tiger nut may be considered a promising candidate for developing novel anticancer agents. However, further molecular studies are necessary to identify the active compounds, clarify the underlying mechanisms of action, and confirm efficacy in in vivo models.

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Declaration of Author Contributions

The authors declare that they have contributed equally to the article, that they have read and approved the final version of the article, ready for publication.

Declaration of Conflicts of Interest

The authors declare no conflict of interest.

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