An In Vitro Examination of The Anticancer Activities of Anthocyanins and Butanol Fractions Extracted from the Rose of Bombax Ceiba Plant Grown in Iraq

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Abstract

As a broad and diverse genus, bombax includes plants native to diverse ecosystems throughout Asia, the Mediterranean, and southern Australia. The purpose of this research was to extract, fractionate, and quantify a few keys physiologically active components of the plant using a Soxhlet apparatus using ethyl acetate and hexane, respectively, due to the polarity differences between these solvents. The second section evaluates the butanol and anthocyanidin fractions for potential anti-cancer efficacy. This is determined by testing the extracts for anti-cancer activity on the MCF7, AMJ13 cell lines at doses ranging from 10 to 300 µg and comparing the extracts' impact to that of a positive control (vincristine) Inhibition of cancer cell proliferation was seen in a statistically significant way (p0.05) when the anthocyanidin fraction of the plant was used. A one-way analysis of variance (ANOVA) revealed that, when compared with the positive control, vincristine, the anthocyanidins fraction of bombax ceiba was more efficient in killing cancer cells than the butanol

Keywords: Anticancer activity, Bombax ceiba, AMJ13, MCF7, Extraction.

1. Introduction

Sadly, cancer has quickly become a global public health concern due to the number of lives it has claimed. Cancer cells were able to invade healthy tissue and spread widely across the body because they could not respond to signals that normally limit cell activity. After establishing a metastatic synapse, cells might migrate to other body parts.[1] The basic defect in carcinogenesis is the uncontrolled and relentless duplication of DNA in cancer cells.[2] The primary distinction between normal and cancerous cells is the cancer cell's lack of growth control when Cancerous and healthy cells are both taken into account over eight million people have lost their lives due to cancer, making it the second biggest cause of mortality worldwide behind cardiovascular disease[3]. Forecasts for the number of new cancer cases grow from 14 million to 21 million, a 60% increase; (cancer deaths are predicted to climb from 4 to 8 billion to 13 billion). Cancer is the leading cause of mortality worldwide, accounting for 1.69 million deaths due to lung diseases, 788 thousand due to liver diseases, 0.5 million due to colorectal cancer, and 0.77 million due to stomach and breast cancers (0.75 million deaths)[4]. Into the Near Future Constant use of drugs and alcohol, a diet low in fruits and vegetables, and a lack of physical activity all contribute to a deteriorating quality of life., Because being overweight is a behavioral and nutritional risk factor that accounts for over a third of all cancer deaths. Using the available information, we located and removed all possible causes of (about 40% of malignancies are curable with the correct preventive measures Receiving a correct diagnosis and treatment promptly Medicines have made it possible to avoid many cases of cancer[5]. Cancer development in both humans and experimental animals has been related to a wide range of environmental and anthropogenic variables. An estimated 15% of all cancers may be traced back to this virus.[6] Bioactive chemicals found in medicinal plants are secondary metabolites with potential anticancer effects; these metabolites serve a variety of functions within the plant, including defense, attraction, and hormone regulation.[7] The Bombacaceae family is comprised of around 22 genera and 150 species, the vast majority of which are found in tropical regions. The Bombax family has sixty different species, the Ceiba family has fifteen, the Durio family has fifteen, and the Salmalia family has ten. The Adansonian (10 species)[8]. The endangered Asian vulture depends critically on Bombax ceiba. extinction. In addition to floss (Kapok) and silk cotton (two different species), Ceiba is used to manufacture a wide range of other commercial commodities[9]. the Bombax ceiba and the pentendra There are a grand total of 55 tropical regions on Earth.[10] The Bombacaceae family, of which the Java kapok6 is a part, produces floss. Bombax ceiba is well known to have analgesic,

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anticancer, antibacterial, gastrointestinal, anti-inflammatory, anti-diabetic, anti-obesity, hepatoprotective, antihyperlipidemic, anti-ulcer, antidiarrheal, antiviral, larvicidal and hypotensive activities[11]. Several in vitro and in vivo investigations have revealed that anthocyanins have the potential to dramatically inhibit the proliferation of cancer cells and stop the formation of tumors. Evidence suggests that anthocyanin-rich pigments might slow down the Carcinogenesis may entail several mechanisms of action, including the suppression of the cyclooxygenase enzyme and the high antioxidant capabilities that may prevent cancer[12]. Anthocyanins stop tumor growth by blocking a kinase that is activated by a mutagen pathway[13]. Anthocyanins, polyphenolic compounds that vary in color from salmon pink to red to violet to dark blue, are responsible for most of the cyanic hues observed in flowers, fruits, leaves, and stems. The classic anthocyanin aglycone is based on a C6-C3-C6 structure.[14] Ninety percent of all anthocyanins are derived from just six anthocyanidins, including pelargonidin, cyanidin, delphinidin, cyanidol, and the slightly different chemical structures of cyanidin, delphinidin, peonidin, petunidin, and malvidin, which only differ by the hydroxylation and methoxylation pattern on their B-rings[15] Using cell viability assays, an IC50 study, and a comparative analysis with positive control, this work intends to evaluate the anticancer potential of different fractions of the ethanolic extract of bombax ceiba on two kinds of breast cancer cells.

2. Material and method

Without additional purification, Merck, BDH, Fluke, Sigma Aldrich Chemicals, and commercial sources were used to obtain all of the chemicals and solvents. ELISA Reader, Human, Germany, Kufa University, Faculty of Medicine. Glass TLC 1020GS, thickness 0.25, 10x20 cm, silica 60.

Plant collection

The leaf and bark were collected from the tree of bombax ceiba in January 2022. there are a few of these trees in Baghdad city in the middle carrot of AL- Salihiyah street. the rose was collected in April 2022 The plant material was stored in a dark and dry place. Then the plant material was crushed and milled into fine particles in order to use in the sequential extractive methods. The College of Science/Herbarium of the Biology Department of Baghdad University verified that the whole bombax ceiba plant was collected in April 2021 from Baghdad Al-Salihiyah.

Preparation of plant extracts Solvent extraction

The anthocyanins in the plant material are extracted using acetone, and then the pigments are further isolated and purified using chloroform partitioning. Introducing chloroform causes a phase transition between the liquid content (including anthocyanin, phenolics, sugars, and organic chemicals that are soluble in water, and the bulk phase, which is made up of pigments such as carotenoids and chlorophyll, lipids, and the immiscible organic solvents substances that are not polar).[16] Using this approach has the benefit of yielding and removing all lipophilic impurities from the extract. Considering there is no concentration phase reduces the potential for pigment degradation due to acid exposure.[16] Using a Soxhlet Continuous Extraction System. By repeatedly heating and chilling the plant extract, its chemical components may be removed. If you need to use an alcohol-like solvent, methanol is a good option since it may be used for a wide variety of tasks. Using solvents that can dissolve both polar and nonpolar chemicals, including methanol, the researchers in this study used the Soxhlet technique to extract all of the valuable compounds, grinding them into a fine powder to increase the surface area and speed up the extraction process. After 24 hours of continuous extraction in a multiple Soxhlet apparatus designed to speed up the process, the powdered plant material was concentrated using a rotary evaporator set to low pressure to remove as much water as possible. The resulting thick extract was then suspended in moderately hot distilled water and fractionated with butanol to yield the final sub extract's composition of butanol solvent[17].

Sample preparation and treatment

bombax cieba butanol and anthocyanidin extracts at 10 mg/ml were used to treat the cell lines AMJ13 and MCF-7. The appropriate amount of test substances was prepared in dimethyl sulfoxide before the experiment. Each cell type was treated with extracts at concentrations of 10, 15, 20, and 300 g/mL diluted in media, then incubated for 72 hours, the optimal treatment time.[18] The produced impact was also compared to that of the conventional medications, vincristine. Each experiment was done a minimum of three times to get the results.

Culture Medium Formulation Without Serum

Liquid media was made by combining g of powdered RPMI-1640 medium with around 900 ml of DDW in a volumetric flask, as directed by the Gibco product manual. Some of the other components include 2 grams of sodium bicarbonate powder and 2.5 milligrams of dry amphotericin B. While stirring frequently, the powder, 1.25 ml of gentamycin stock solution, and 1 mL of streptomycin stock' solution were added. With the use of NaOH, HCl, and a PH meter, we were able to keep the PH steady at 4.2, and we were able to increase the volume of the solution to 1 liter by adding DW until we achieved the desired concentration. Filters with micron grades of 0.4 and 0.2 M were used to clean the water. The medium was kept at 4 degrees after it was ready to be utilized.[19]

Making Research-Ready Cell Lines of AMJ13 and MCF-7

Both cell lines arrived in two distinct frozen vials, and they were both kind donations from the Babylon medical cell culture facility. At 37 degrees, 25 ml of growth medium was added to each flask, and the cultures were kept there.[20]

Thawing of frozen cell lines

In order to conduct the experiment, the frozen cell line must first be thawed. After being taken out of the Nitrogen container, the frozen cell line vial was put in a 37 degrees Celsius water bath on a sterile glass plate with Sterile DW for the cultivation process. After a little delay, the hot cells were moved to a sterile centrifuge. At room temperature, put 10 mL of culture medium in a test tube. Using centrifugation, the cells at the bottom of the tube were collected, and then suspended in 5 mL of growth medium before being added to a Sterilized culture flask and incubated at 37°C. Altering the growing conditions for plants.[21]

Methods of Cell Isolation and Passage

This process requires the protein-digesting enzyme trypsin in order to be successful. With the intention of freeing the monolayer cells that have been stuck. We will get a culture flask ready when it is needed. Furthermore the following processes are involved: After the cells had formed a monolayer, the old medium was pulled in and discarded..[22]The attached monolayer of cells was washed with PBS solution equivalent to about half the volume of the growing medium, and this procedure may be repeated as many times as required depending on the degree of cell damage. well-known as a follower of that belief system. Following a brief wash, 3 ml of a warm trypsin-EDTA solution was applied to the cells. After the medium was added, the culture flask was gently shaken four or five times. Disrupt the cell monolayer. Lastly, step four included putting the flask into an incubator to wait for the outcomes to be determined. The time required to separate cells that have clustered together is minimal. Nine to ten minutes after that. The cells were taken out of the incubator on day five, and 10 ml of growth medium was added to the flask to suspend them. The FBS present in the culture medium, however, will stop the trypsin from rejoining the separated cells. Either two separate sterile flasks or a micro-plate were used to further develop the resulting solution..[23]

The Research Strategy and Its Elements, Assessed

in this research, assays were used to figure out the effects of changing various study factors. The AMJ13 and MCF-7 cell lines were treated with butanol and anthocyanide extracts, respectively. Two different time-varying extract dosages were tested. Inside of 24 hours, at the latest. The formation of a colony of proliferating cells in a flask is referred to as confluence. Prior to entering the exponential phase, the cells were harvested and diluted to a concentration of 5X 105 cell / ml in growth medium. Total of eight 96-well culture plates were utilized. equipped with a nutrient solution for cell development and waiting to be planted the adjacent sixty micro wells remained in place and were filled with DW to keep the incubator at a constant humidity level. After the cells had grown to 80% confluence, the test was diluted multiple times and put into the wells. Substances chemically similar to those used in the examples below.[24]

Cytotoxicity activity

Viability of AMJ 13, MCF-7 cell line after treatment with two fractions of bombax ceiba plus positive control

The viability percentage is represented as mean ± standard error of the mean (SEM). Viability can be estimated when calibrating the result of IC50 (subtract the % of inhibition from the concentration of drug. It was shown from the data the viability of MCF-7 is less significant when compared with the viability of AMJ 13when treated with a different extract and compare the effect with positive control[25].(**figures 1,2**) and **Table (1,2)**.

Table(1)) % of the viability of MCF7 cells when treated with different fractions of the bombax ceiba extract and vincristine as positive cotrol with different conc. Started from 10[c]µg/ml to 300[c]µg/mL.

[c]µg/ml	% of the viability of MCF7 CELLS when treated with butanol E			% of the viability of MCF7 cell when treated with anthocyanidine			% of the viability of MCF7 CELLS when treated with vincristine		
10	96.1	96.3	96.6	80.7	81.6	80.7	69.7	71.5	73.6
15	97.2	96.4	95.2	94.6	75.3	76.6	58.7	56.4	57.6
20	96.3	96.1	96.2	70.6	68.4	66.8	45.7	50.6	49.8
30	94.3	94.7	94.2	60.6	58.5	55.8	39.7	37.6	36.8
40	91.6	91.6	92.4	56.2	48.1	50.6	29.7	28.2	32.8
50	91.6	90.7	90.6	41.7	44.8	41.6	22.6	23.7	24.8
100	80.1	82.2	78.2	36.7	38.4	33.7	11.6	19.8	17.4
150	70.5	74.4	78.2	28.5	26.3	31.8	10.7	6.5	5.8
200	67.2	60.6	66.4	33.8	32.8	28.5	2.6	3.8	5.1
300	55.1	42.1	55.4	36.6	34.7	25.3	9.8	7.7	0.7

Table(2)) % of the viability of AMJ13 cells when treated with different fractions of the bombax ceiba extract and vincristine as positive cotrol with different conc. Started from 10[c]µg/ml to 300[c]µg/mL.									
[c]µg/ml	% of the viability of AMJ13 CELLS when treated with butanol E			% of the viability of AMJ13 CELLS when treated with anthocyanide			% of the viability of AMJ13 CELLS when treated with vincristine		
10	96.4	92.8	96.1	83.2	78.6	77.9	64.6	66.2	60.9
15	96.4	95.8	92.8	69.8	77.3	66.3	55.4	60.2	53.3
20	93.6	91.8	92.7	40.8	61.7	63.2	44.7	48.4	55.3
30	85.4	68.4	86.4	50.8	55.7	49.5	38.7	34.8	37.9
40	32.8	68.4	69.5	48.6	46.2	43.2	33.8	25.8	28.7
50	50.8	56.4	55.4	40.7	39.2	43.2	31.8	28.7	24.8
100	26.4	34.3	32.7	32.7	28.4	29.7	9.8	23.8	32.8
150	3.7	11.6	9.7	18.5	22.8	39.7	0	10.4	18.2
200	0.8	1.5	2.7	27.3	26.1	25.2	9.5	0.9	3.8
300	0	0	0	23.8	30.6	27.4	0.1	0	0

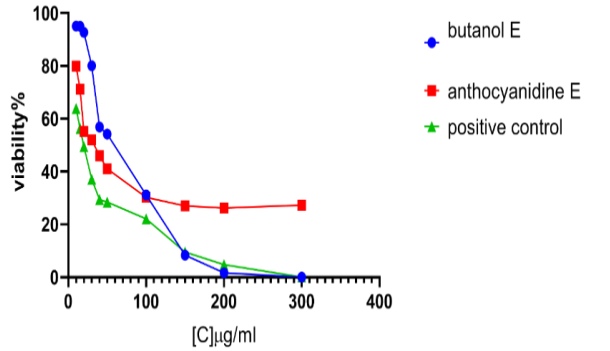

Figure (1) viability of AMJ-13 cell line after treated with bombax extracts and positive control.

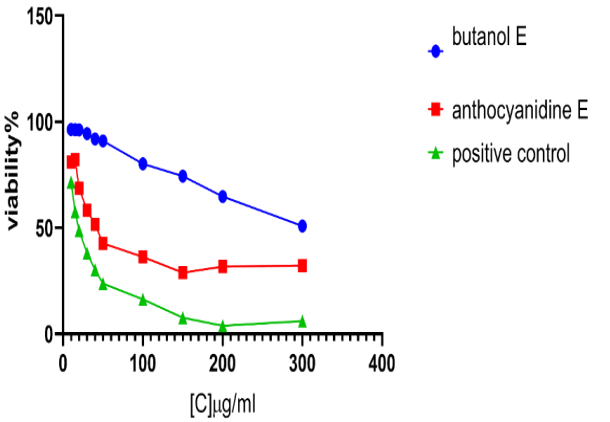

Figure (2) viability of MCF-7 cell line after treated with bombax extracts and positive control.

Determination of IC50 and % inhibition of AMJ 13, MCF-7 cell line after treatment with two fractions of bombax ceiba plus positive control

The anticancer effect of different fractions (butanol,anthocyanidine) of bombax ceiba extract that compared with the activity of positive control(vincristine) was assessed by MTT method The results of the MTT method are shown in Figure (3,5), Table (3,4), revealed specific anticancer activity of the extract towards breast cancer cell line AMJ13 and MCF-7 cell line The plant extract showed a substantial ($p < 0.05$) cytotoxicity towards AMJ13 cells in a dose-dependent manner (Figure 4,6) The IC50 for butanol fraction on AMJ13 cells was calculated to be 64.178±3.214 µg/mL whereas IC-50 values of anthocyanide fraction on AMJ13 cells was calculated to be 34.815 ± 1.478 µg/ ml and finally IC50 values of positive control (vincristine) on AMJ13 cells was 21.253 ± 0.327 µg/ mL, while The IC50 for butanol fraction on MCF-7 breast cancer cell line was calculated to be 260.328 ± 0 µg/ mL whereas IC50 values of anthocyanide fraction on MCF-7 cells was calculated to be 41.319 ± 2.689 µg/mL and finally IC50 values of positive control (vincristine) on MCF-7cells was 21.253±0.327. The bombax ceiba extract (anthocyanidine) showed the most potent cytotoxic effects against the AMJ13 and MCF-7cancer cell line, with an IC50 value of 34.815 ± 1.478, 41.319 ± 2.689 µg/mL.

Table (3) % of the inhibition of MCF7 cells when treated with different fractions of the bombax ceiba extract and vincristine as positive cotrol with different conc. Started from 10[c]µg/ml to 300[c]µg/mL.									
[c]µg/ml	% Of the inhibition of MCF7 CELLS when treated with butanol E			% Of the inhibition of MCF7 CELLS when treated with anthocyanide			% Of the inhibition of MCF7 CELLS when treated with vincristine		
10	3.9	3.7	3.4	19.3	18.4	19.3	30.3	32.5	34.4
15	2.8	3.6	4.8	5.4	24.7	23.4	41.3	43.4	42.4
20	3.7	3.9	4.8	29.4	31.6	33.2	54.3	49.4	50.2
30	5.7	5.3	5.8	39.4	41.5	44.2	60.3	62.4	63.2
40	8.4	8.4	7.6	43.8	51.9	49.4	70.3	71.2	67.8
50	8.4	9.3	9.4	58.3	55.2	58.4	73.4	76.3	75.2
100	19.9	17.8	21.8	63.3	61.6	66.3	88.4	80.2	82.6
150	29.5	25.6	21.8	71.5	73.7	68.2	89.3	93.5	94.2
200	32.8	39.4	33.6	66.2	67.2	71.5	97.4	96.2	94.9
300	44.9	57.9	44.6	63.4	65.3	74.7	90.2	92.3	99.3

Table(4) % of the inhibition of AMJ13 cells when treated with different fractions of the bombax ceiba extract and vincristine as positive cotrol with different conc. Started from 10[c]µg/ml to 300[c]µg/mL.									
[c]µg/ml	% of the inhibition of AMJ13 CELLS when treated with butanol E			% of the inhibition of AMJ13 CELLS when treated with anthocyanide			% of the inhibition of AMJ13 CELLS when treated with vincristine		
10	3.6	7.2	3.9	16.8	21.4	22.1	35.4	33.8	39.1
15	6.8	6.2	7.2	30.2	22.7	33.7	44.6	39.8	46.7
20	6.4	8.2	7.3	39.2	38.3	36.8	55.3	51.6	44.7
30	14.6	13.7	13.6	49.2	44.3	50.5	61.3	65.2	62.1
40	27.2	31.6	29.5	51.4	53.8	56.8	66.2	74.2	71.3
50	49.2	43.6	44.6	59.3	60.2	56.8	78.2	71.3	75.2
100	73.6	65.7	77.3	67.3	71.6	70.3	90.2	86.2	77.2
150	96.3	88.4	90.3	81.5	77.2	60.3	100.3	89.6	81.8
200	99.2	98.5	97.3	72.7	73.9	74.8	90.5	99.1	96.2
300	109.3	110.1	112.3	76.8	69.4	72.6	99.9	103.4	102.9

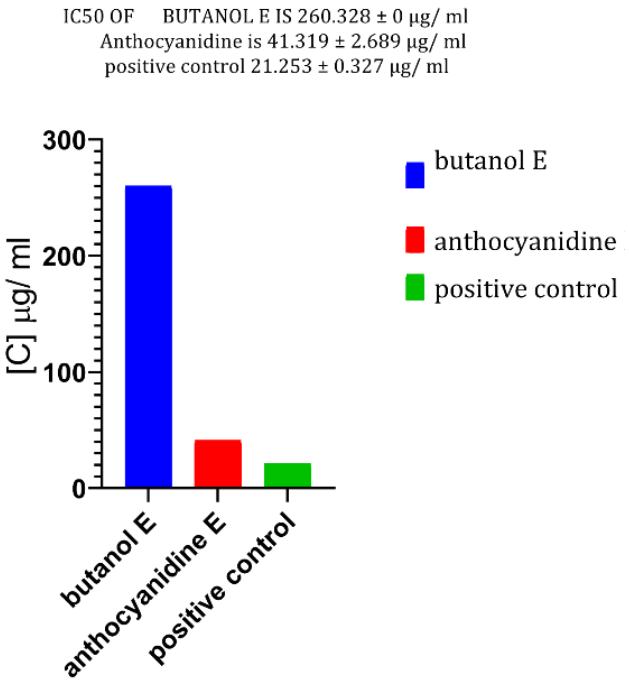

*Figure (3) % of inhibition of the MCF-7 CELLS and IC50 estimation when treated with two different **fractions** of bombax ceiba extract and also with positive control.*

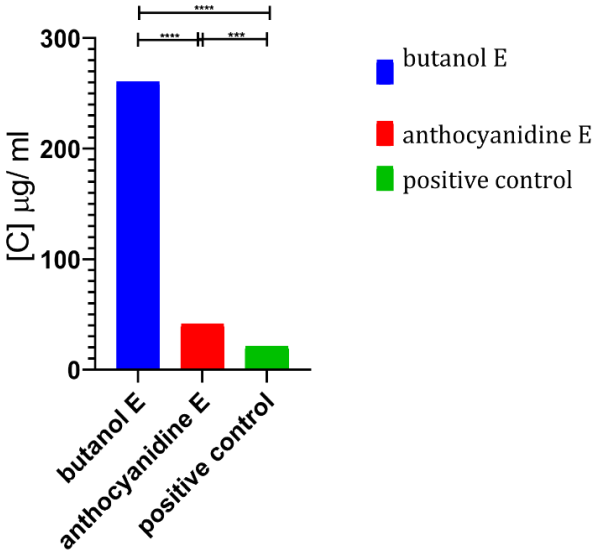

Figure (4)significance of the effect of different treatments on the MCF 7 cells when treated with two different fractions of bombax ceiba extract and also with positive control.

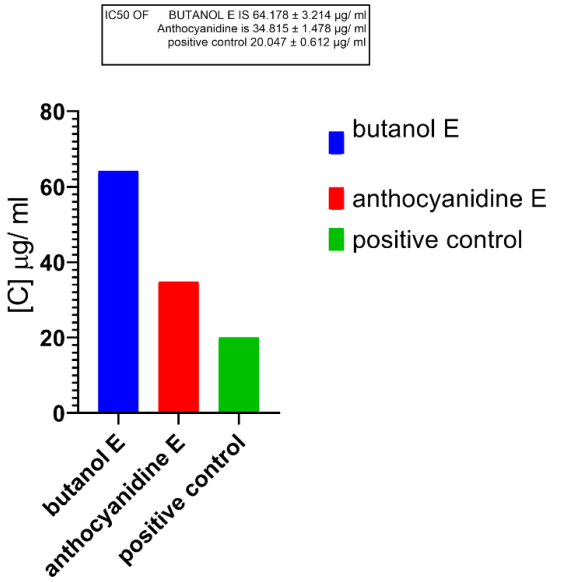

Figure (5) % of inhibition of the AMJ 13 CELLS and IC50 estimation when treated with two different fractions of bombax ceiba extract and also with positive control.

3. Discussion

Cytotoxic effect of butanol fraction

Cytotoxic effect of butanol fraction on MCF-7 cell line show anti-cancer activity due to the presence of antioxidant compounds that favor this fraction of plant but the activity shows more significant when compare this activity on AMJ13 cell line in competition with the MCF-7 cell line (Figure 4, 6).

Cytotoxic effect of anthocyanide fraction

Several in vitro and in vivo investigations have revealed that anthocyanins have the potential to dramatically inhibit the proliferation of cancer cells and stop the formation of tumors. Evidence suggests that anthocyanin-rich pigments might slow down the Carcinogenesis may entail several mechanisms of action, including the suppression of the cyclooxygenase enzyme and the high antioxidant capabilities that may prevent cancer[12]Cytotoxic effect of anthocyanide fraction shows high significance in both type cancer cell ($P \leq 0.05$) as in figure (4,6) with high correlation between dose and response (IC50) when compared with positive control (vincristine)

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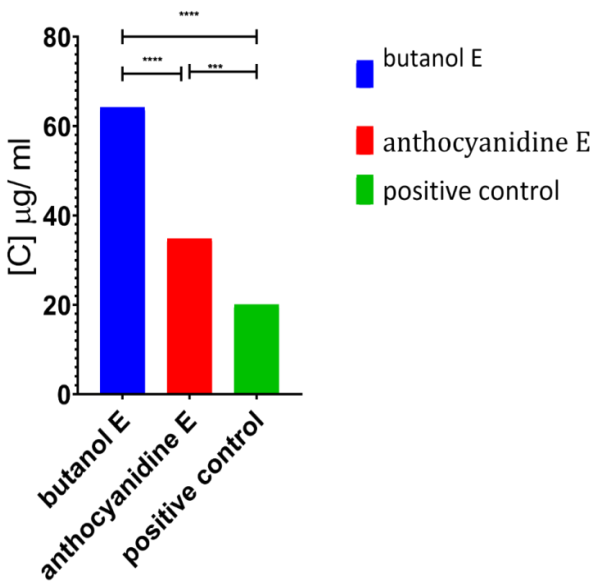

Figure (6) significance of the effect of different treatments on the AMJ 13cells when treated with two different fractions of bombax ceiba extract and also with positive control.

4. Conclusions

he following inferences may be made from the data we have collected: one result of phytochemical analysis of Iraqi bombax ceiba, which led to the isolation of chemicals with potential medicinal use. We also need to focus on the rose as an element of the root. The bulk of the results of this investigation are consistent with data on the isolated compounds that has been gathered by scientists from throughout the world. The percentage of butanol. rose anthocyanide fraction showed the greatest promise in in vitro tests examining its anti-cancer effects. Anthocyanin-rich pigments may explain anti-cancer effectiveness by inhibiting the Inhibition of the cyclooxygenase enzyme and potent antioxidant properties are two potential modes of action in carcinogenesis.

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