# In Vitro Anticancer Activity of Different Fractions of Bombax Ceiba Plant Cultivated in Iraq That Compared with Vincristine as A Positive Control

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**Abstract**
The bombax genus is a large and taxonomically complex one that consists of different species present in Asia, Mediterranean Australia, and southern Asia regions. This study is concerned with the extraction, fractionation, and estimation of some known biologically important constituents from the plant extraction of these constituents was carried out by Soxhlet apparatus, the active constituents according to the differences in their polarities using, ethyl acetate and hexane. The second part is the assessment of the possible anti-cancer activity of the hexane-ethylacetate fractions This is done by anti-cancer activity on the MCF7, AMJ13 cell lines using different concentrations of extracts starting from 10µg to 300 µg and comparing the effect of extracts with the positive control (vincristine) The results showed that the hexane, fraction from the plant show inhibition of the growth of cancer in a manner showed that significant of the experiment; ($p<0.05$). After detection of the most active fraction of bombax ceiba detection of inhibitory concentration (IC50) was done one-way Anova test showed that hexane fraction has a more effective effect on cancer cells than ethyl acetate fraction when compared with positive control which was vincristine.

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

## 1. Introduction

Cancer's quick rise to a worldwide public health issue is largely attributable to the number of lives it has sadly cut short. Because they were unable to react to signals that regulate cell activity, cancer cells were able to penetrate healthy tissue and circulated all around the body. cells then spread to other organs through the metastatic synapse. Constant and unchecked multiplication of the cancer cell's DNA is the primary aberration in carcinogenesis. The primary distinction between normal and cancerous cells is the cancer cell's lack of growth control when Both cancerous and healthy cells are considered. Second, only to cardiovascular disease, cancer is a leading killer globally causing over eight million deaths. Projections of new cancer cases rise from 14 million to 21 million, a sixty percent increase;1 (cancer deaths, from 4 to 8 billion to thirteen billion. In terms of the reason of death, cancer is the most frequent including lung disease (1.69 million fatalities), liver disease (788 thousand deaths), and colorectal cancer ( 0.5 million deaths) (0.7 million deaths), stomach and breast (0.75 million deaths).2 For the foreseeable future Drug and alcohol abuse, a sluggish poor consumption of fruit and vegetables, little exercise, and a high body mass index an important behavioral and dietary risk factor that accounts for almost a third of all deaths from cancer. Using the accessible data, we identified and eliminated potential sources of (about 40%) of malignancies are curable with the right preventative measures. Diagnosis at an early stage and receiving Many malignancies are now preventable thanks to medicines Multiple factors, including radiation, viruses, chemicals, and environmental pollutants, have been linked to cancer growth in both people and experimental animals. This virus is responsible for around fifteen percent (15%) of all malignant neoplasms3. The advance of research in medicinal plants discovers the different bioactive compounds that act as secondary metabolites which have different roles inside the plant (such as defense, attractive, and hormonal roles) that have the potential anticancer activity.About 22 genera and 150 species live in the tropics and make up the family Bombacaceae. Bombax (with sixty species), Ceiba (with fifteen), Durio (with fifteen), and Salmalia (with ten species) are among the most numerous plant families.4 Adansonia (10 species). Production of match splints and matchboxes is also part of the company's repertoire. The Asian vulture, a threatened species, relies heavily on Bombax ceiba for its survival. extinction. Ceiba is used to produce a variety of other marketable goods, including floss (Kapok) and silk cotton two distinct species,5 the pentendra and the Bombax ceiba.L total of 55 tropical areas may be found around the globe. The Bombacaceae family, which includes the floss-making Java kapok6. Bombax ceiba is well known to have analgesic, anticancer, antibacterial, gastrointestinal, anti-inflammatory, anti-diabetic, anti-obesity, hepatoprotective, antihyperlipidemic, anti-ulcer, antidiarrheal, antiviral, larvicidal and hypotensive activities7. The current research largely

doi.org/10.31838/hiv23.01.91 Received: 21.09.22, Revised: 13.10.22, Accepted: 20.12.22

aims to assess the anticancer potential for different fractions of the ethanolic extract of bombax ceiba on two types of breast cancer cells through employing various assay methods such as, cell viability, IC50 study, and comparable study with positive control.

## 2. Material and method

All chemicals and solvents were obtained from Merck, BDH, Fluke, Sigma Aldrich chemicals company and commercial sources and were used without further purification. Glass TLC 1020GS with silica 60, thickness 0.25, size 10x20cm. ELISA Reader, Kufa university, Faculty of medicine, Human, Germany.

### Plant collection Method

The leaf and bark were collected from the tree of bombax ceiba. there are a few of these trees in Baghdad city in the middle carrot of AL- Salihiyah street. 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

Using the soxhlet continuous extraction method. Chemical components may be extracted from plants using this technique by continuously heating and cooling the lants extract. Using a solvent like alcohol, such as methanol, methanol is a reliable all-purpose solvent. This study extracted all of the useful compounds by the Soxhlet method for an extended period of time in solvents that are able to dissolve both polar and nonpolar compounds which is methanol, make a fine powder that increases the surface area and speeds up the extraction process. The crushed plant material was then extracted with 85% alcohol with 15% water, and the powdered plant material was concentrated (after continual extraction for 24 hr in the multiple Soxhlet apparatus for speeding of extraction), and concentrated by using a rotary evaporator under reduced pressure until we get the thick extract, then suspended with moderately hot distilled water then fractionated with different solvent started from low to high polarity by using aseparatory funnel.[8] The -MeOH extracts were then crudely divided into sub extracts based on their polarity. Starting with n-hexane, nonpolar, intermediate, and polar extracts were produced. The sub-extracts of n-hexane were mixed and evaporated to dryness. Then, ethyl acetate fractionation is performed.[9]

### Sample preparation and treatment

The cell lines AMJ13 and MCF-7 were treated with 10 mg/ml of bombax cieba ethyl acetate and hexane extracts. Prior to the experiment, desired quantities of test chemicals were produced in dimethyl sulfoxide. The reactant mixtures were diluted with medium, and cells were treated with various concentrations of the extract (10,15,20,200,300g/mL) and incubated for 72 hours, which was the best treatment duration of the extracts in each of the cell lines.[10] 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.

### Formulation of Serum-Free Culture Medium

Following the instructions in the Gibco product handbook, liquid medium was prepared by mixing g of powdered RPMI-1640 medium with around 900 ml of DDW in a volumetric flask. Other ingredients include 2.5 mg dry amphotericin B and 2 g sodium bicarbonate powder. The powder, 1.25 ml of gentamycin stock solution, and 1 mL of streptomycin stock` solution were added while being stirred constantly. The PH was maintained at 4.2 with the use of NaOH, HCl, and a PH meter, and the solution's volume was brought up to 1 liter by adding DW until it reached that concentration. The water was purified by passing it through filters with respective micron ratings of 0.4 and 0.2 M. Once the medium was ready to be used, it was stored at 4 degrees Celsius.[11]

### Preparing the AMJ13 and MCF-7 Cell Lines for Research

Both cell lines were kind gifts from the Babylon medical cell culture facility and were sent in two different frozen vials. Each was cultured in a flask holding 25 ml of growth media at 37 degrees Celsius.[12]

### Cell line defrosting

Thawing the frozen cell line is a prerequisite to the experiment. Culturing, following removal from the Nitrogen container, the frozen cell line vial was placed in a 37°C water bath on a sterile glass plate with Sterile DW. A short wait later, the molten cells were transferred to a clean centrifuge. Ten milliliters of culture media in a test tube at room temperature. Centrifugation The cells that had sunk to the bottom of the tube were removed and suspended in 5 mL of growth media before the mixture was transferred to a Sterilized culture flask and placed in a 37°C incubator. Changing the soil or medium in which plants are grown.[13]

### Procedures for Isolating and Subculturing Cells

Harvesting is reliant on the protein-digesting enzyme trypsin. With the aim of dislodging the adhered monolayer cells and releasing them. When it's time, we'll prepare a culture flask. and the following procedures are involved: old media was sucked in and thrown away as the cells reached a monolayer.14 A PBS solution containing about half the volume of the growth medium used was used to clean the monolayer of connected cells, and this process may

be repeated as many as necessary depending on the extent of the cells' damage. is well recognized as an adherent. In this case, we washed the cells and then added 3 ml of a warm trypsin-EDTA solution. Thereafter, the culture flask was gently shaking four or five times after the addition. Rip apart the monolayer of cells. Four, the flask was placed in an incubator to await final results. Separation of clustered cells does not need much more time than that. Following that, nine to ten minutes. At the end of day five, the flask containing the cells was removed` from the incubator, and` 10 ml of growth media was added` to the flask in order to suspend the` cells. However, the FBS in the culture media will prevent the trypsin from recombining the divided cells. The resultant suspension was then either sub-cultivated into two separate sterile flasks or cultured on a micro-plate.15

## Evaluations of the Research Plan and Its Elements

The impact of the study's parameters was determined by assays. Extracts from ethyl acetate (EA) and hexane (HE) were used to treat AMJ13 and MCF-7 cell cultures. EA and HE doses that varied over time were tried out. As soon as possible, within 24 hours. When a colony of multiplying cells in a flask forms, the process is called "confluence." The cells were collected and diluted in growth media at a concentration of 5X 105 cell / ml before they reached the exponential phase. eight 96-well culture plates were used. stocked with a cell-growth media solution and ready for planting The sixty micro wells next to them were left unmoved and were filled with DW to maintain humidity in the incubator. When cell growth reached 80%, the test was diluted several times and placed in the wells. Chemicals of the kind used in the demonstrations below.[16]

## 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 estimated on AMJ 13 cells is found more sophisticated than estimated from MCF -7, the 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 13 when treated with a different extract and compare the effect with positive control, (Figures 1,2) and Table (1,2)17.

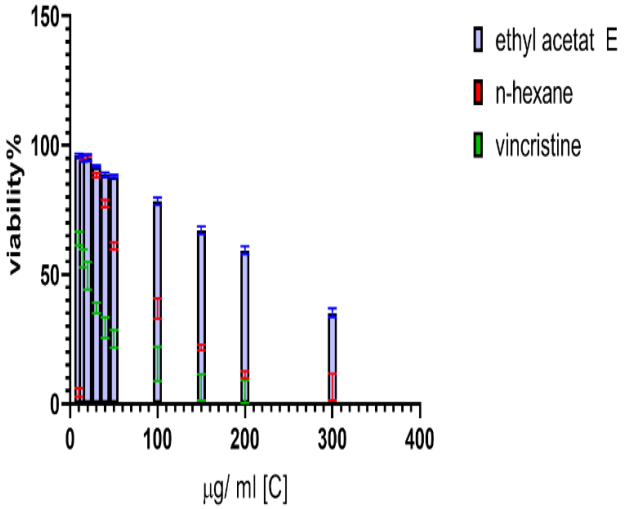


*Figure (1) viability of AMJ-13 cell line after treated with bombax extracts and positive control.*

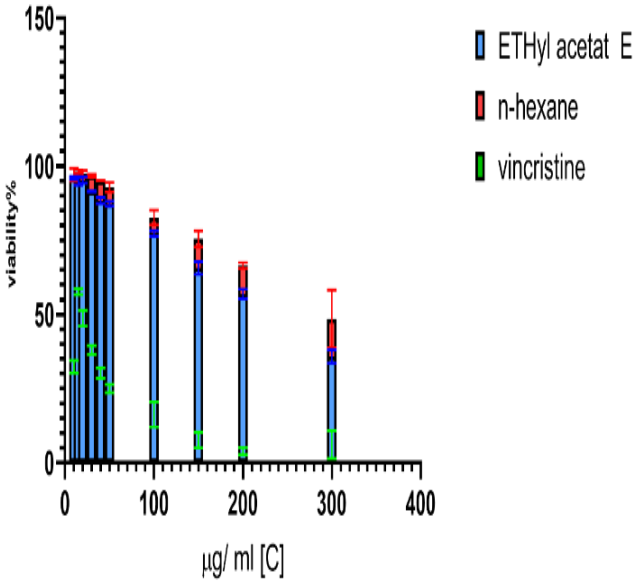


*Figure (2) viability of MCF-7 cell line after treated with bombax extracts and positive control.*

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 ethyl acetate E | | | % of the viability of MCF7 CELLS when treated with hexane E | | | % of the viability of MCF7 CELLS when treated with vincristine | | |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 95.7 | 96.7 | 95.9 | 94.7 | 97.6 | 99 | 30.3 | 32.5 | 34.4 |
| 15 | 96.3 | 94.4 | 93.9 | 98.5 | 98.7 | 97.4 | 58.7 | 56.6 | 57.6 |
| 20 | 95.2 | 96.6 | 94.4 | 98.6 | 97.4 | 96.3 | 45.7 | 50.6 | 49.8 |
| 30 | 91.6 | 91.7 | 91.4 | 96.8 | 97.3 | 96.4 | 39.7 | 37.6 | 36.8 |
| 40 | 88.4 | 87.4 | 89.4 | 95.3 | 95.1 | 95.3 | 29.7 | 28.8 | 32.2 |
| 50 | 87.5 | 86.4 | 88.1 | 94.7 | 91.6 | 92.6 | 26.6 | 23.7 | 24.8 |
| 100 | 77.4 | 76.3 | 78.3 | 82.6 | 85.3 | 80.3 | 11.6 | 19.8 | 17.4 |
| 150 | 63.3 | 66.2 | 67.6 | 77.6 | 76.6 | 72.4 | 10.7 | 6.5 | 5.8 |
| 200 | 58.6 | 55.4 | 56.8 | 66.6 | 65.4 | 67.4 | 2.6 | 3.8 | 5.1 |
| 300 | 38.4 | 34.7 | 34.3 | 59.7 | 44.5 | 41.3 | 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 ethyl acetate E | | | | % of the viability of AMJ13 CELLS when treated with hexane E | | % of the viability of AMJ13 CELLS when treated with vincristine | | |
| 10 | 95.7 | 95.4 | 96.8 | 3.2 | 6.2 | 3.6 | 64.6 | 66.2 | 60.9 |
| 15 | 96.2 | 93.5 | 94.9 | 94.7 | 94.4 | 93.8 | 55.4 | 60.2 | 53.3 |
| 20 | 95.8 | 93.8 | 96.1 | 95.7 | 93.7 | 94.9 | 44.7 | 48.4 | 55.3 |
| 30 | 91.5 | 91.8 | 92.4 | 88.6 | 89.2 | 87.4 | 38.7 | 34.8 | 37.9 |
| 40 | 89.3 | 87.5 | 88.7 | 78.7 | 75.8 | 77.9 | 33.8 | 25.8 | 28.7 |
| 50 | 88.6 | 87.4 | 88.1 | 59.8 | 62.6 | 60.8 | 21.8 | 28.7 | 24.8 |
| 100 | 79.7 | 78.6 | 76.8 | 37.5 | 40.5 | 32.7 | 9.8 | 13.8 | 22.8 |
| 150 | 66.8 | 68.7 | 65.8 | 20.6 | 22.7 | 21.9 | 0.7 | 10.4 | 8.2 |
| 200 | 57.6 | 60.7 | 59.8 | 12.8 | 10.1 | 10.9 | 9.5 | 0.9 | 3.8 |
| 300 | 36.7 | 35.8 | 33.3 | 8.6 | 10.4 | 0.7 | 0 | 0 | 0 |

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

The anticancer effect of different fractions (EA,H) 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 ethyl acetate fraction on AMJ13 cells was calculated to be 236.284 ± 5.116 µg/ ml whereas IC-50 values of hexane fraction on AMJ13 cells was calculated to be 77.697 ±2.322 µg/ ml and finally IC50 values of positive control (vincristine) on AMJ13 cells was 20.047 ± 0.612 µg/ ml , while The IC50 for ethyl acetate fraction on MCF-7 breast cancer cell line was calculated to be 274.100±2.245 µg/ mL whereas IC50 values of hexane fraction on MCF-7 cells was calculated to be 234.987 ± 5.423µg/ml and finally IC50 values of positive control (vincristine) on MCF-7cells was 21.253 ± 0.327. The bombax ceiba leaf extract (hexane fraction) showed the most potent cytotoxic effects against the AMJ13 cancer cell line, with an IC50 value of 77.697 ± 2.322 µg/ml (Fig. 2b). In contrast, the extract showed no noticeable cytotoxic effect against MCF7, 234.987 ± 5.423 , with extremely high IC50 values for these cells .18

| 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. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| % of the inhibition of MCF7 CELLS when treated with vincristine | | | % of the inhibition of MCF7 CELLS when treated with hexane E | | | % of the inhibition of MCF7 CELLS when treated with ethyl acetate E | | | [c]µg/ml |
| 34.4 | 32.5 | 30.3 | 3.9 | 3.8 | 4.1 | 2.3 | 3.3 | 2.4 | 10 |
| 42.4 | 43.4 | 41.3 | 5.3 | 4.9 | 5.2 | 3.6 | 2.4 | 2.9 | 15 |
| 50.2 | 49.4 | 54.3 | 4.8 | 5.1 | 5.2 | 3.4 | 2.9 | 2.3 | 20 |
| 63.2 | 62.4 | 60.3 | 8.7 | 7.9 | 8.3 | 4.9 | 5.1 | 4.3 | 30 |
| 67.8 | 71.2 | 70.3 | 13.4 | 10.9 | 11.2 | 6.9 | 7.3 | 8.4 | 40 |
| 75.2 | 76.3 | 73.4 | 12.7 | 14.4 | 13.4 | 7.2 | 8.8 | 9.4 | 50 |
| 82.6 | 80.2 | 88.4 | 20.2 | 23.6 | 21.4 | 16.9 | 16.4 | 18.3 | 100 |
| 94.2 | 93.5 | 89.3 | 31.4 | 37.3 | 33.2 | 22.5 | 24.6 | 29.3 | 150 |
| 94.9 | 96.2 | 97.4 | 41.5 | 44.4 | 40.2 | 33.6 | 34.2 | 39.3 | 200 |
| 99.3 | 92.3 | 90.2 | 65.9 | 61.4 | 63.4 | 49.6 | 55.7 | 43.5 | 300 |

| 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 ethyl acetate E | | | % of the inhibition of AMJ13 CELLS when treated with hexane E | | | % of the inhibition of AMJ13 CELLS when treated with vincristine | | |
| 10 | 4.3 | 4.6 | 3.2 | 3.2 | 6.2 | 3.6 | 35.4 | 33.8 | 39.1 |
| 15 | 3.8 | 6.5 | 5.1 | 5.3 | 5.6 | 6.2 | 44.6 | 39.8 | 46.7 |
| 20 | 4.2 | 6.2 | 3.9 | 4.3 | 6.7 | 5.1 | 55.3 | 51.6 | 44.7 |
| 30 | 8.5 | 8.2 | 7.6 | 11.4 | 10.8 | 12.6 | 61.3 | 65.2 | 62.1 |
| 40 | 10.7 | 12.5 | 11.3 | 21.3 | 24.2 | 22.1 | 66.2 | 74.2 | 71.3 |
| 50 | 11.4 | 12.6 | 11.9 | 40.2 | 37.4 | 39.2 | 78.2 | 71.3 | 75.2 |
| 100 | 20.3 | 21.4 | 23.2 | 62.5 | 59.5 | 66.3 | 90.2 | 86.2 | 77.2 |
| 150 | 33.2 | 31.3 | 34.2 | 79.4 | 77.3 | 78.1 | 100. | 89.6 | 81.8 |
| 200 | 42.4 | 39.3 | 40.2 | 87.2 | 88.9 | 89.1 | 90.5 | 99.1 | 96.2 |
| 300 | 63.3 | 64.2 | 66.7 | 91.4 | 89.6 | 99.3 | 99.9 | 103.4 | 102.9 |

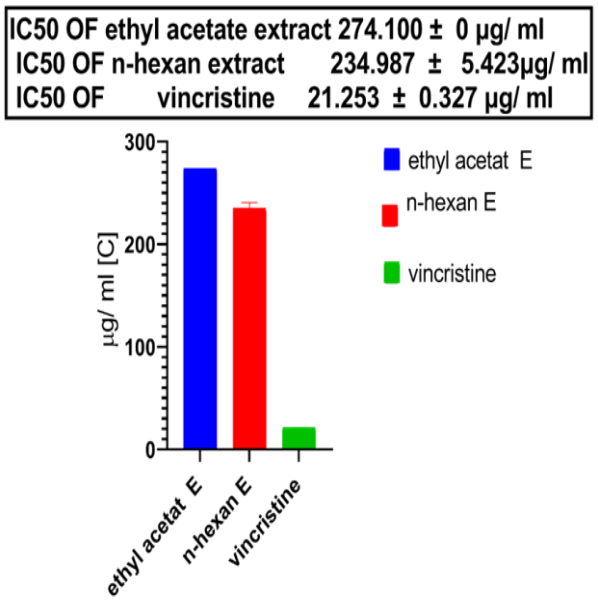


*Figure (3) % of inhibition of the MCV7 CELLS and IC50 estimation when treated with two different **fractions** of bombax ceiba extract and also with positive control.*

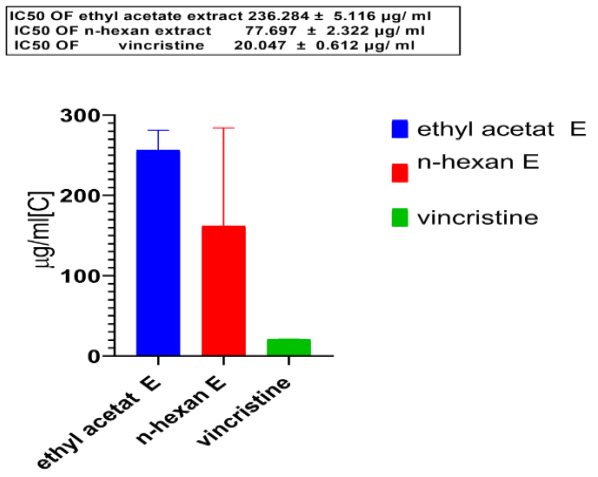


*Figure (5) % of inhibition of the AMJ 13 CELLS and IC50 estimation when treated with two different fractions of bombax ceiba extract and also with positive control.*

## 3. Discussion

### Cytotoxic effect of ethyl acetate fraction

Cytotoxic effect of ethyl acetate fraction on MCF-7 cell line show less significant when compared with hexane fraction on the same type of cell line (figure 4) while on the AMJ13 cell line the significance of ethyl acetate are better ($P \leq 0.05$) but the correlation between dose and response weaker than that of hexane on the same type cell line (figure 6)

### Cytotoxic effect of hexane fraction

Cytotoxic effect of hexane 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) this is due to high content of terpine specify lupeol which has anti-cancer activity and more prominent in hexane fraction than in the ethyl acetate fraction.

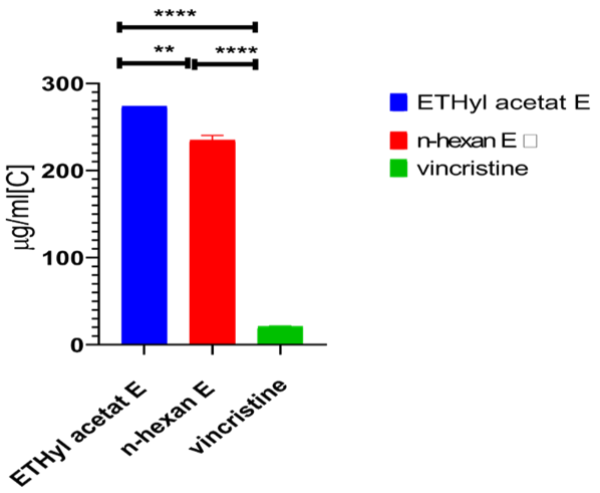


*Figure (4)significance of the effect of different treatments on the MCF 7 cells when treated with two different fractions of bombax ceiba extract and also with positive control.*

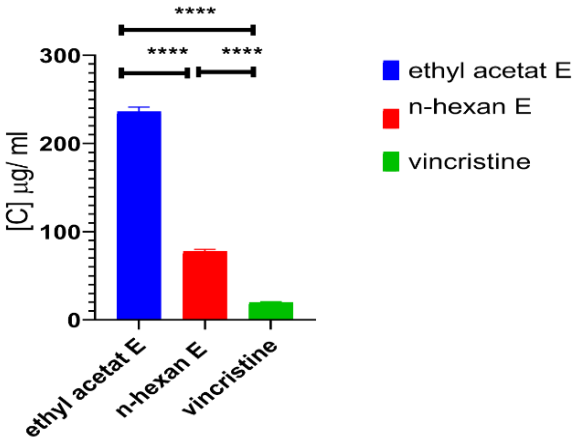


*Figure (6) significance of the effect of different treatments on the AMJ 13cells when treated with two different fractions of bombax ceiba extract and also with positive control.*

## 4. Conclusions

Based on the obtained results, the following points may be concluded: one finding from the phytochemical screening of Iraqi bombax ceiba, where compounds were isolated from the plant's which has a number of useful compounds. Another component of the the root, rose, needs our attention. The majority of this study's findings corroborate previous information on the separated chemicals from other international researchers. The hexane content. displayed potent anti-cancer activity in vivo studies, with the ethyl acetate fraction of leaves and bark sections showing the most promise effect. Anti-cancer efficacy may be explained by the presence of terpenes, phenolic compounds, fatty acids, and other phytochemicals in the active fractions. IC50 of hexane extract represented as atoxic when compared with positive control IC50.

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