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Investigating the anti-proliferation activity of Conocarpus erectus Against breast tumor cells in vitro

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ABSTRACT

The methanolic extract of Conocarpus erectus was tested in vitro on cancerous cell lines AMJ, MCF7, Cal51, and MDMBA and normal mouse embryonic fibroblast (MEF) cell line as a comparison. Morphological study for exposed cells was done by examination under phase-contrast inverted microscope. High-performance liquid chromatography (HPLC) was used to assay the concentrations of each component the phenols or flavonoids in the Conocarpus erectus methanol extract. Cell proliferation was reduced with concentrations after exposure time 72 h on AMJ and MDAMB cell line. Of contact there was fluctuation in the cell response to different concentrations of the extract on MCF7 and Cal51 cell line, compared with no effect on normal cell line (MEF). In conclusion, Conocarpus erectus has anticancer activity and may be considered a new source to use it in breast cancer therapy for future researches.

Keywords: Conocarpus erectus for breast tumor

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INTRODUCTION

The name "cancerwas used for the first time by Hippocrates, father of western medicine, who applied Greek words carcinoma and Karakinos to describe tumour" [1]. Malignancy is an uncontrolled development of anomalous cells in the body [2]. Typically, meiosis and cell demise strategies happen to ensure stable state of tissues in adjusted state [3]. Carcinogenesis is a multistage or multi instrument strategy. The underlying phase of malignant growth incorporates irreversible cell changes. The advancement stage is clonal multiplication of cells and movement stage incorporates forceful and metastatic period of infection.

Medical procedure, chemotherapy, and radiotherapy are viewed as the most widely recognized techniques for disease therapy, albeit these therapy strategies are not generally helpful and the clinical outcomes are not worthy [4].

Home grown medications incorporate plants, natural edifices, and natural items or plant or even a blend of plants which were utilized thousand years before creating present day drugs. They are likewise utilized these days [5]. Natural plants are utilized everywhere on the world in various strategies both allopathic and customary frameworks [6]. *Conocarpus erectus*, commonly called buttonwood or button mangrove [2], is a mangrove shrub in the family Combretaceae. This species grows on shorelines in tropical and subtropical regions around the world.

C. erectus is usually a dense multiple-trunked shrub, 1–4 m (3.3–13.1 ft) tall, but can grow into a tree up to 20 m (66 ft) or taller, with a trunk up to 1 m (3.3 ft) in diameter. The leaves are alternately arranged, simple and oblong. The fruits are a cluster of red to brown, small scaly, two-winged cone-like seeds (from which the common names derive).

In Iraq there are no studies about its effect *in vivo* and *in vitro* on tumor cells; therefore, this study was to investing the active compound with active agama tumor cells as *in vitro* study.

MATERIALS AND METHODS

Collection of Plant materials:

Collection of Conocarpus leaves were obtained from Al-mesayab town at 60 Km south of Baghdad. The plant was identified and authenticated by the National herb of ministry of Agriculture of Iraq.

Plant Extraction

Preparation of crude extract the powder samples (50 g) were extracted with methanol solvent (500 ml) by using soxhlet apparatus for 72 h. After complete extraction, the methanol solvent was evaporated by using rotary evaporator (Yamato, Rotary Evaporator model – RE – 801) under reduced pressure to obtain methanol crude extract.

High-Performance liquid chromatography (HPLC) analysis

HPLC technique mainly utilizes a column that holds filing material (the stationary phase), a pump that transfers the mobile phase through the column, and the detector that show the retention times of the molecules. The retention time differs depending on the interactions between the stationary phase, the molecules that analyzed and the solvent used (7,8).

Column : 3 micrometer particle size (50*4.6 mm 1.D) shimpack C-18 , mobile phase : 0.1% phosphoric acid : acetonitrile (52:24, V/V), Detection UV set at 285nm.
Flow rate 1.5 mL / min .
Temp : 25 c .
The concentration for each compound was quantitatively determined by comparison the peak area of the standard with that of the sample

Cell Lines:

The breast cancer cell lines AMJ13 (as a locally established cell line) (9), MCF7, MDAMB, and CAL51 were cultured in a MEM medium (US biological, USA) with 10% fetal bovine serum (FBS) (Capricorn- Scientific, Germany), 100 units/mL penicillin, and 100 µg/mL streptomycin. While the mouse embryonic fibroblast (MEF) cell line (as a control regular cell line) were cultured in an RPMI-1640 medium (US biological, USA) supplemented with 10% fetal bovine serum (FBS) (Capricorn- Scientific, Germany), 100 units/mL penicillin, and 100 µg/mL streptomycin. After culturing these cell lines were incubated at 37°C.

These cells are routinely surveyed for standard development qualities, and they are consistently validated. All cell lines were refined as disciple blended monolayers and kept up at 37°C in a humidified climate of 5% CO2. Cells were reaped after brief trypsinization with trypsin-EDTA (Capricorn-Logical, Germany).

Cytotoxicity assays:

To decide the cell-murdering impact of C. erectus extricate, Precious stone violet cell suitability test was led on 96-well plates (Santa Clause Cruze Biotechnology, USA), Human Bosom malignant growth cell lines (AMJ13, MCF7, MDAMB and CAL51), just as should be expected mouse early stage cells (MEF) were cultivated at 7000 cells/well after 24hr or blended monolayer is accomplished, cells were treated with C. erectus remove at 9 overlap weakenings from (4000µg to 15.6µg) in culture media. Cell practicality was estimated at 72hrs of presentation by eliminating the medium, including 50 µL of Gem violet stain (Sigma Aldrich, USA), and brooding for 20 minutes at 37°C. Subsequent to eliminating the stain, it washed with PBS and afterward dried. At last, the receptiveness was resolved on a microplate peruser (Biochrom, UK) at 492 nm (test frequency); the examine was acted in three-fold. Results were communicated as the rate multiplication regarding vehicle-treated cells (10).

RESULTS

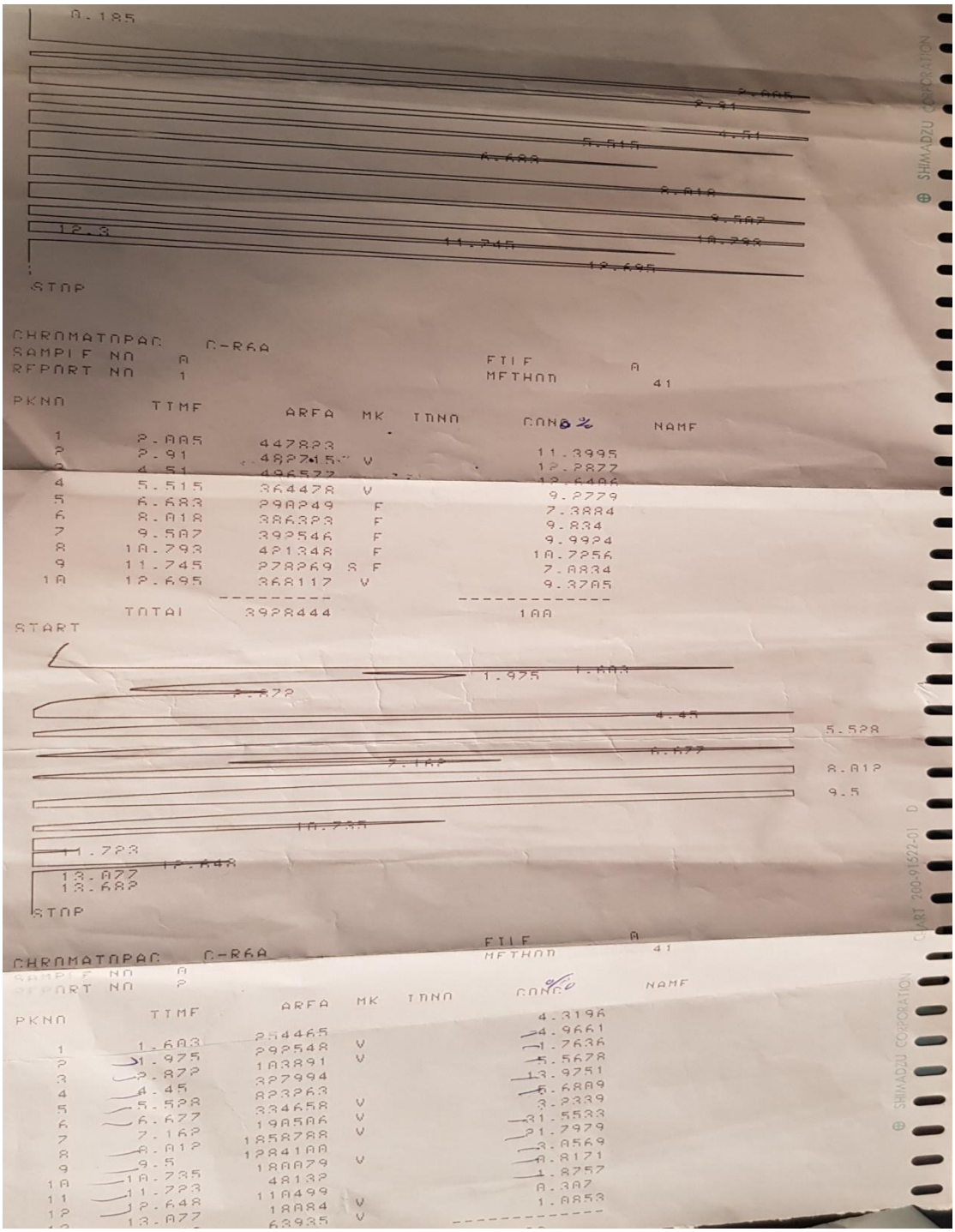
HPLC of *Conocarpus erectus*:

The results at table (1) and diagram (1) were referred to the plant extract was contained different types of flavonoid glycosides, but the concentrations of most important of these glycosides such as catechin, quercetin -3- a-d (1-8) galic acid , qurecetin -3-a-b-d- glucoside and Kaempferol were read to 9.40 , 13.15, 15.50 and 28.50% as percentage of total extract respectively.

Table (1): HPLC results of *C. erectus* leaves extract.

Standard	Rt	Cons.	Area	Sample	Rt	Area	Cons.
Gqllic acid	1.92	3.14	154304	Gqllic acid	1.83	35250	2.74
Catechin	3.50	5.62	137487	Catechin	3.47	122583	9.40
Quercetin -3-0-B-D- glucoside (1-6) galic acid	5.54	8.92	151324	Quercetin -3-0-B-D- glucoside (1-6) galic acid	5.50	176178	13.51
Quercetin -3-0-B-D- glucoside	6.25	10.06	138114	Quercetin -3-0-B-D- glucoside	6.22	2072083	15.50
Kaempferol-3-0-rutinoside	6.91	11.13	128675	Kaempferol-3-0-rutinoside	6.90	58927	28.50
Kaempferol -3-0-B-D glncoside	8.08	13.01	137352	Kaempferol -3-0-B-D glncoside	8.06	58927	4.51
Kaempferol	8.91	14.34	144254	Kaempferol	9.03	89182	6.88
Qnercetin	9.83	15.82	141629	Qnercetin	9.87	119740	9.14
Apigenin	11.15	17.96	129231	Apigenin	11.15	128167	9.82

Diagram (1): The HPLC analysis of C. *erectus*



Cytotoxicity assay:

Selective cytotoxic effect of *Conocarpus erectus* extract:

The current study investigated the selective cytotoxic effect of the *C. erectus* extract in some breast cancer cells. In our experiments, we used four human breast cancer cell lines (AMJ13, MCF7, MDAMB, and CAL51), and normal mouse embryonic cells (MEF) in our study. All cell lines were exposed to C. erectus extract at 9 fold concentrations started from 4000 to 15.6 µg/mL for 72 h and cytotoxicity was determined using crystal violet assays.

The results of this study showed that the extract induced cell death only in all the cancer cells used in this study, whereas no changes were observed in the viability of the normal embryonic cells MEF.

The results showed that C. erectus extract (with all concentration used) have no effect on normal cell line (MEF) and with no variation of cell density after exposure to all concentration of extract (as shown in figure 1).

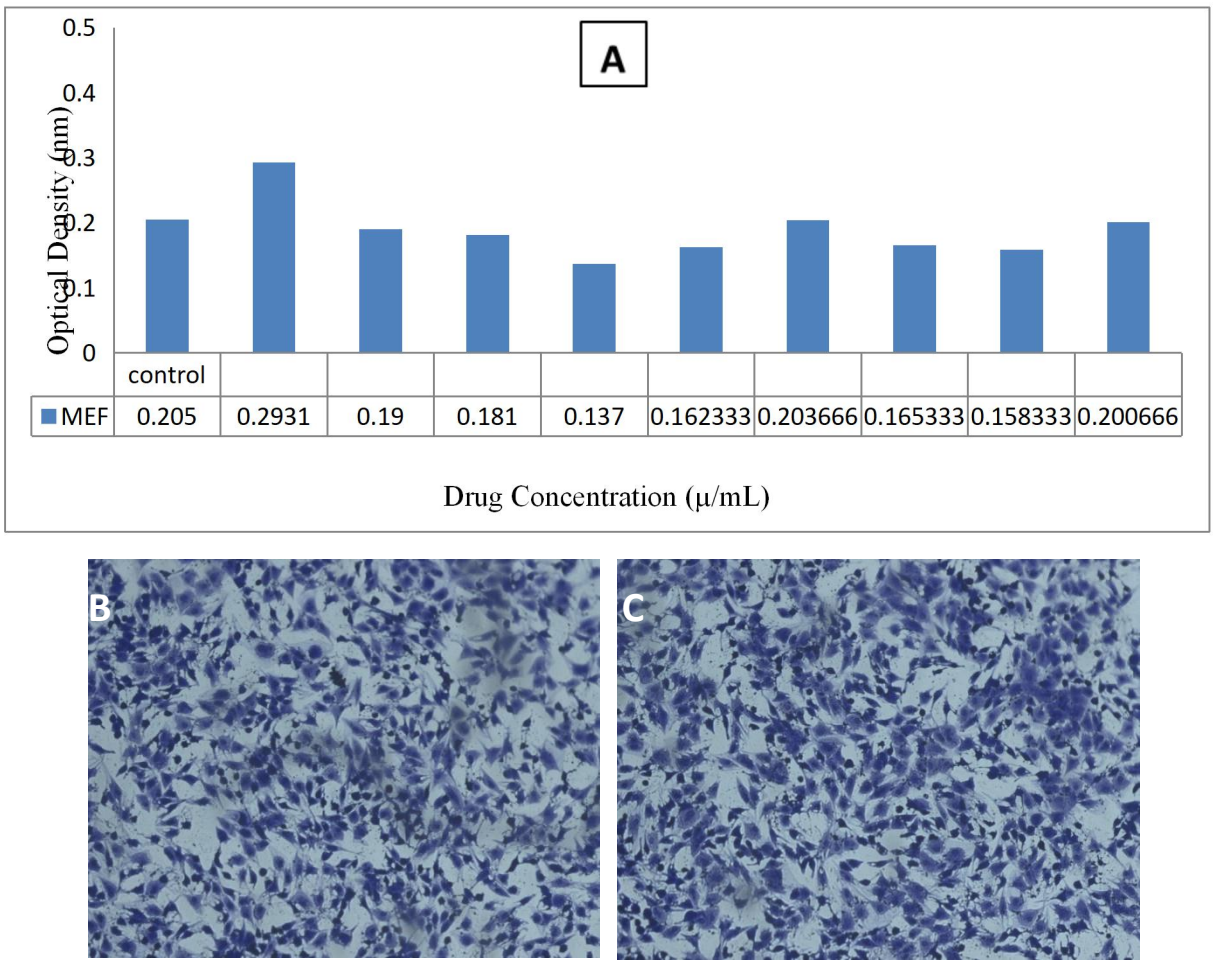
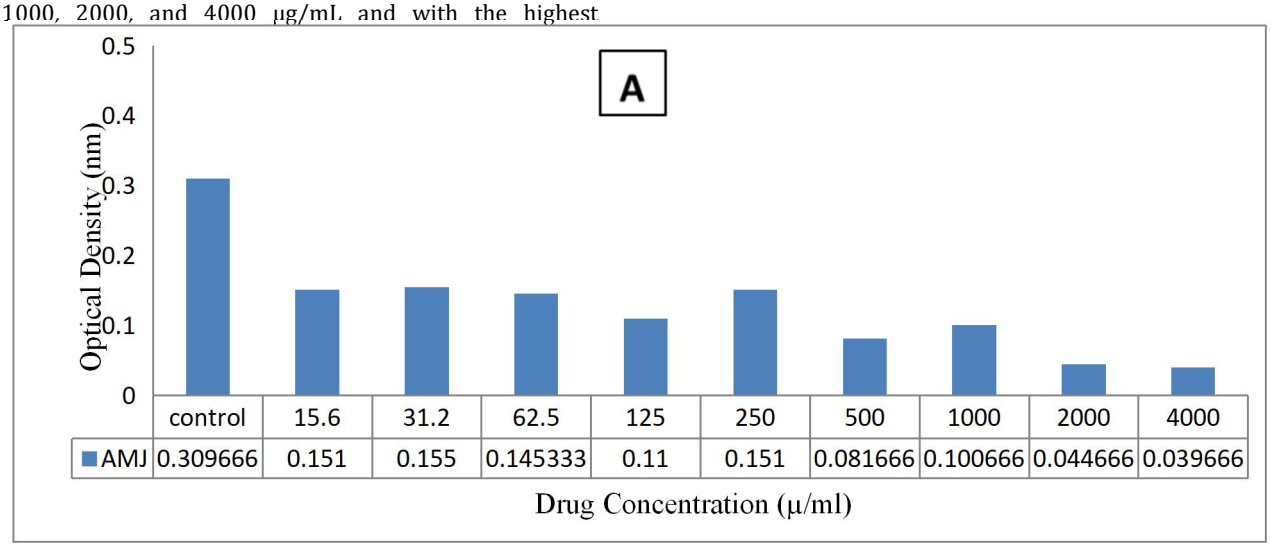


Figure (1): (A) showed the effect of *C. erectus* extract on normal cell line MEF from 15.9- 4000 µg/ml. No variation of cell density with the highest concentration of 4000 µg/mL (B) compared with control (without extract) (C) of MEF cell line.

The cancer cell lines AMJ, and MDAMB have cell death induction and decreased on cell density after exposed to *C. erectus* extract which increased with concentration 500, 1000, 2000, and 4000 µg/mL and with the highest concentration in 4000 µg/mL in AMJ cell line (figure 2), and with 125 and 250 µg/mL in MDAMB cell line (figure 3).



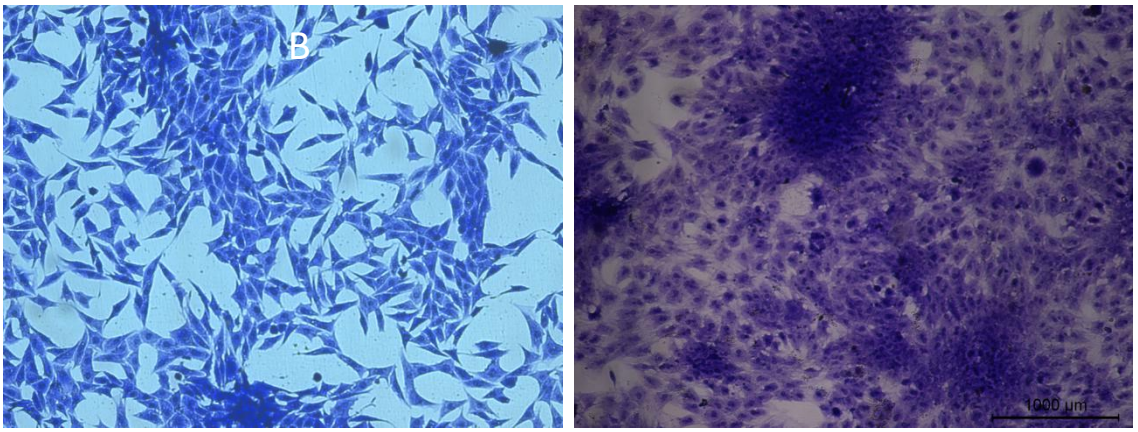
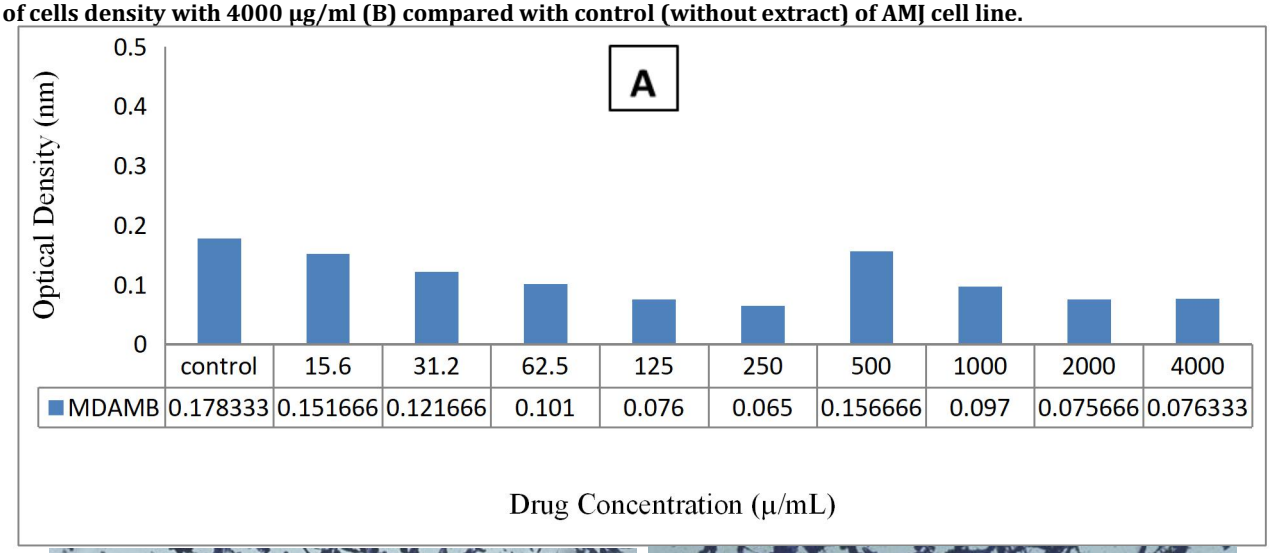


Figure (2): (A) showed the effect of *C. erectus* extract on AMJ cell line from 15.9- 4000 µg/mL. (B & C) The variation of cells density with 4000 µg/ml (B) compared with control (without extract) of AMJ cell line.



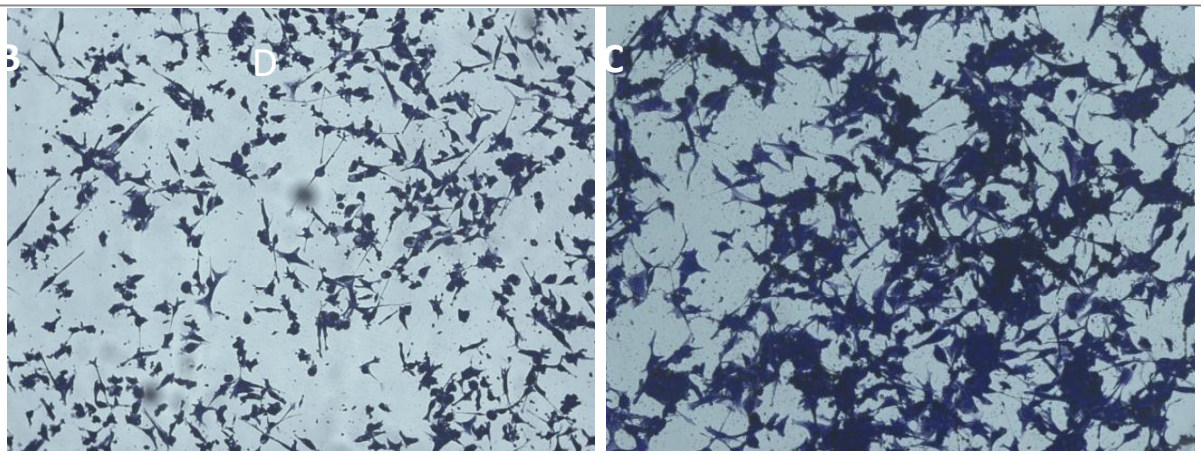


Figure (3): (A) showed the effect of *C. erectus* extract on MDAMB cell line from 15.9- 4000 µg/ml. The variation of cells density with 250 µg/ml (B) compared with control (without extract) (C) of MDAMB cell lines.

While the result of both MCF7 and Cal 51 cell line showed that there is a noticeable fluctuation in the cell response to different concentrations of the extract, which may occur in such plant extracts reaching to the best concentration (1000 µg/ml in MCF7 cell line) (2000 µg/mL in Cal 51) (figure 4, and 5).

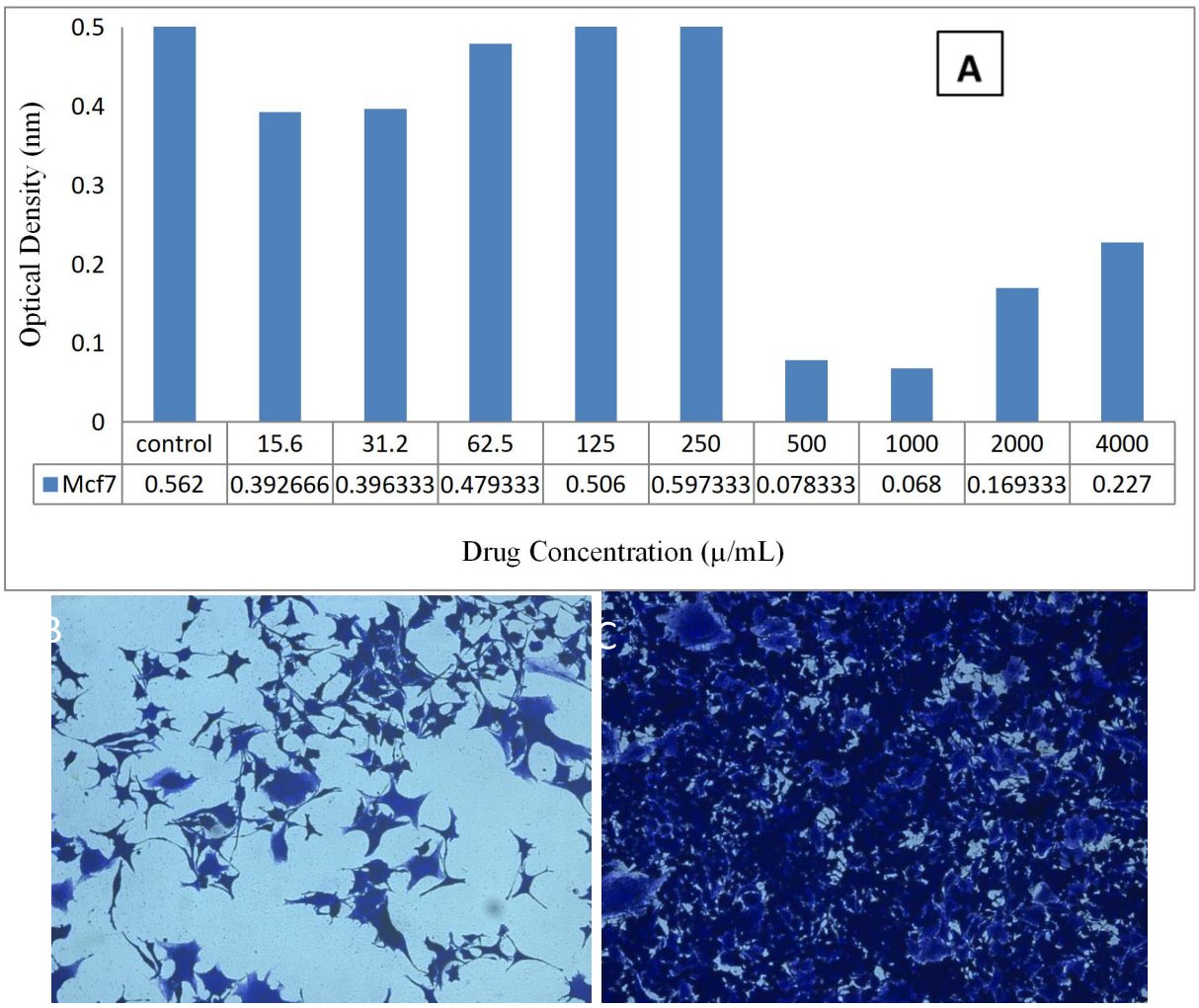
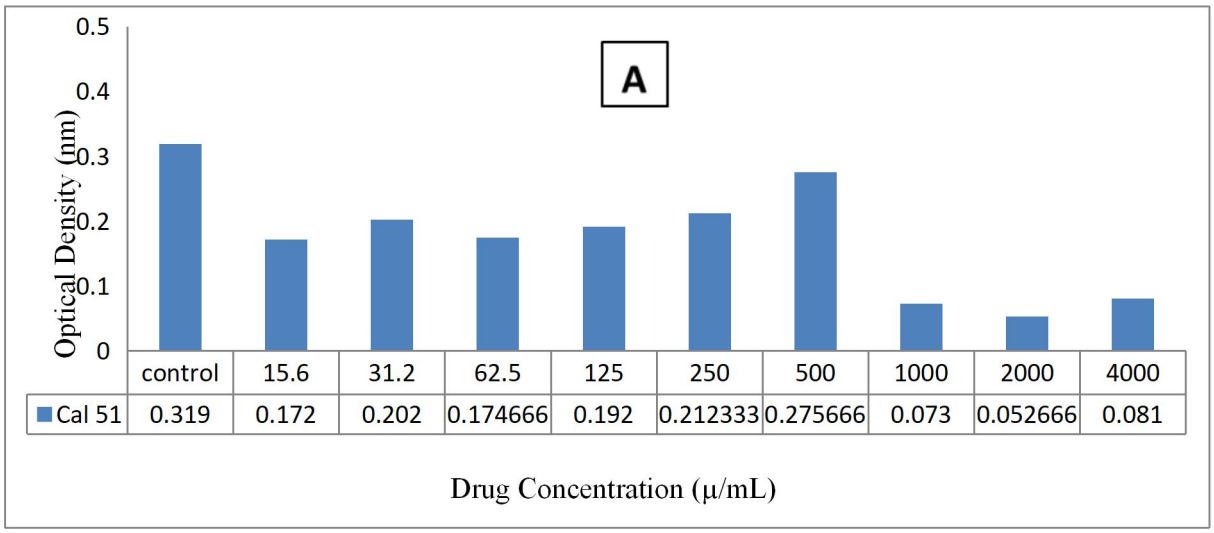


Figure (4): (A) showed the effect of *C. erectus* extract on MCF7 cell line from 15.9- 4000 µg/mL. The variation of cell density with 1000 µg/mL (B) compared with control (without extract) (C) of MCF7 cell line.



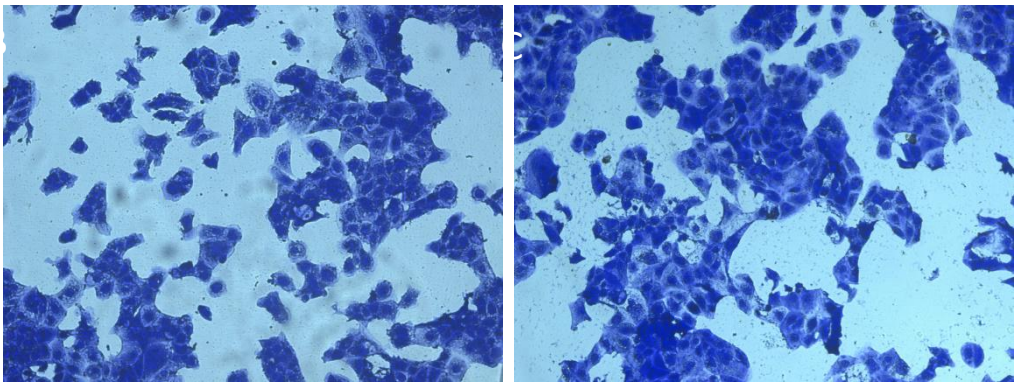


Figure (5): (A) showed the effect of *C. erectus* extract on Cal51 cell line from 15.9- 4000 µg/mL. (B): Showed the variation of cell density with 4000 µg/mL, compared with control (without extract) (C) of Cal51 cell line.

DISCUSSION

Flavonoids have a place with bunch substances with variable phenolic structure and are found in natural product, vegetables, grains, bark, roots, stems, blossoms, tea and wine (11). Their common items were known by their helpful consequences for wellbeing; flavonoids were disengaged as compelling mixes. More than 4000 assortments of flavonoids have been distinguished, a large number of which are dependable for the alluring shades of blossoms, natural product, and leaves (12). Nonetheless, it has been generally known for quite a long time that subordinates of plant source have a expansive range of natural movement (13). An important effect of flavonoids is the scavenging of oxygen-derived free radicals in vitro experimental systems also showed anti-carcinogenic properties (14).

Working mechanisms: Antioxidative effect

The best-portrayed property of pretty much every gathering of flavonoids is their ability to go about as cancer prevention agents the flarones and chaechins appear to be the most remarkable flavonoids for ensuring the body against receptive oxygen species body cells and tissues are ceaselessly undermined by the harm brought about by free revolutionaries and responsive oxygen species, which are created during ordinary oxygen digestion or are incited by exogenous harm (15,16). The instruments and the succession of functions by which free extremists meddle with cell capacities are not completely seen, however one of the most significant functions is by all accounts lipid peroxidation, which brings about cell film harm. the cell harm causes a move in the net difference in the cell changing the osmotic weight, prompting growing and in the long run cell passing. Free revolutionaries can pull in different incendiary arbiters, adding to general fiery reaction and tissue harm. To shield themselves from receptive oxygen species, living life forms (17).

The antioxidant defense mechanisms of the body include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, but also nonenzymatic counterparts such as glutathione, ascorbic acid, and tocopherol. The increased production of reactive oxygen species during injury results in consumption and depletion of the endogenous scavenging compounds. flavonoids may have an additive effect on the endogenous scavenging compounds. Flavonoids are polyphenolic compounds that occur in foods and plant origin. Their proposed protective role in tumor development may prevail especially in the intestinal tract due to direct exposure of intestinal epithelial to these dietary ingredients. In addition to selected compounds we assessed whether they included apoptosis by determining a caspase-3 activation. Studies on the dose-dependent effects on the flavonoids showed antiproliferative activity of all compounds with EC has ante proliferative effect in differ caners.

In conclusion, the methanolic extracts of *Conocarpus erectus* containing different types of flavonoids *(*catechin, quercetin -3- a-d (1-8) galic acid , qurecetin -3-a-b-d-glucoside and Kaempferol) having anticancer activity and may considered a promising source to use it in breast cancer therapy for future researches.

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REFERENCES

1. Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E. et al. Natural compounds for cancer treatment and prevention. Pharmacol Res . 2009;59(6):365–78. [PubMed]
2. Jena J. A Study on Natural Anticancer Plants. Int J Pharmaceut Chem Sci 2012;1(1):365-8.
3. Dhorajiya BD, Patel JR, Malani MH, Dholakiya BZ. Plant product (R)- Roscovitine valuable inhibitor of CDKs as An anti-cancer agent. Der Pharmacia Sinica 2012;3(1):131-43.
4. Yang G, Li X, Li X, Wang L, Li J, Song X, et al. Traditional chinese medicine in cancer care: a review of case series published in the chinese literature. Evid Based Complement Alternat Med 2012;2012:751046.
5. Pal SK, Shukla Y. Herbal medicine: current status and the future. Asian Pac J Cancer Prev 2003;4(4):281-8.
6. Smith-Hall C, Larsen HO, Pouliot M. People, plants and health: a conceptual framework for assessing changes in medicinal plant consumption. J Ethnobiol Ethnomed 2012;8:43.
7. Abbas, k.mahdi, A .H and Ibrahim S. Abbas .Effect of humic and salicylic acides on oil yield and flavonoids glycosides of safflower (Curthumus tinctoorius L .)as medicinal plant grown in Iraq . 2018 . in J. of pharmacentical sciences and research . vol .9. Issue .5 .
8. Ibrahim ,S.Abbas . the study of biometric and volatile oil quantity of sag plant (salvia officinalis L.) as a medicinal plant affected by nitrogen andphosphorus fertilizers ,Int .J pharmacy , and pharmacetial , Vol . 6, issue , 6. 2014 .

9. Al-Shammari AM, Alshami MA, Umran MA, Almukhtar AA, Yaseen NY, Raad K, et al. Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient. Breast Cancer : Targets and Therapy. 2015;7:223-30.
10. 10- Al-Khafaji AS, Ahmed M, Firas S. Photodynamic action of low power He-Ne laser on photosensitized human Hep-2 and AMN3 cell lines with hematoporphyrin derivative in vitro. Iraqi J of Canc and Med Gen. 2010;3(1):54-60.
11. 11- Ibrahim ,S. Abbas . chemical detection for active compounds , some of mineral elements in leave of (Ruta grovelones L.) and influence of alcoholic , aqueous extraction on growth of microorganism . 2011 .J .of wassit for science and medicine . vol .(4):1.
12. 12-Ibrahim S.Abbas ,Effect of nitrogen and phosphorus fertilization on growth parameters with detection the total phenolic content of nettle (Urtica diocia L.) plant .2015 .vol . 4 issue 8 .
13. 13-Ibrahim S, aljubory , thamer m . jasiem , Sara R. Baderden , noor m . Nasser . cultivation and comparison study of pharmacoguostical and phytochemical properties of white and black mustard plants grown under semi-arid conditions in Iraq . 2015 , vol .3 , issue 12 . 1045 -1048 .
14. 14-Shihab Hattab mutlag , Maha noori hamad , Ibrahim salih abbas . the evaluation of ethyl aetate fraction of cressa cretica effect on mitotic index and micronucleus frequency in mice . 2017 Int .J . pharm . sci . rev . res 45 (11). No .28 , 147 – 150 .
15. 15-Noor .S. jaafar, maha N . hamad ., Ibrahim , S . Abbas ., Iman ,S . Jaafar Qualitative phtochemical composition between flavonoids and phenolic acides contents of leaves and fruits of melia a2 edarach (family : melia C eae) cultivated in iraq raqHPLC . 2016 . vol .8 . issue 10 .
16. 16--Jung ws , chung IM , Rim SH , Rim my , Ahmed A , praveen N .Invitro antioxidant activity , total phenolics and flaronoids from celery (Apium gravevlens) leaves . Journal of medicinal plant research 2011 ; 5 (32) ; 7022 – 7030 .
17. 17-Abbas Rashams , mahdi Abed Hamza , Ibrahim S. Abbas . Effect of humic and salicylic acids on oil yield and flavonoid glycosides of safflowes (carthamus tinctorius L) grown in Iraq . IJPSR. 2018; 9 (5): p. 2100-2014