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Cytotoxic Effect of methanolic extract of *Morenga oleifera* leaves on two cancer Cell Lines in vitro

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The methanolic crude extract of *Morenga oleifera* leaves was prepared using soxhlet apparatus. To assess the cytotoxic effects of plant extract against female intestine cells of Albino mice (L20B) and human breast cancer (MCF7) cell lines various concentrations of *Morenga oleifera* extracts (12.5, 6.25, 3.125, 1.56 /mg/ml) were used to treatment of both cell lines after 24 h. and 48 h. of exposure time, for cytotoxic effect studies. According to the results the hot methanolic *Morenga* extract exhibited remarkable cytotoxicity against two examined cancer cell lines with the maximum percentage of growth inhibition (37%) and (59.6%) which observed in the treatment with 12.5 mg/ml against MCF7 cell line and L20B cell line, respectively. These percentages were increased after 48 hr. of incubation to (56%) against MCF7cell line and (66%) against L20B cell line. Finally, GC mass analysis for crude plant leaves extract was done to recognize the most active chemical constituents and the result of GC mass analysis for this extract proved the presence of 15 biologically active compounds included nine compounds that have antioxidant and anticancer activities. This research has shown that the *Morenga oleifera* leaves had potent anticancer activity and it could serve as a possible source of drug against cancer. In addition, the current study gave the first report that provided the presence of anticancer activity in crude methanolic extract from *Morenga oleifera* leaves against L20B cell line.

Keywords: ***Morenga oleifera*** **, alternative chemotherapy, cancer, MMT assay**

INTRODUCTION

Cancer is the mortality causing disease worldwide. World Cancer Research Fund,(2007) and World Health Organization (WHO) ,(2008)published a reports indicate that the incidence of tumor still increasing particularly due to diet style, effects of environments and infections of cancer causing viruses. The artificial medications that uses for cancer treatment are beyond the reach of common cancer patients because of cost aspect ,Herbal medicines have a crucial role in the of cancer prevention and dealing and medicinal plants are usually available and comparatively economical (Sundaram*et al*., 2011)Herbal medicine is still the most widely distributed source for principle health care of about 65- 80% of the world's population, primarily in developing countries, may due to better compatibility with the human body, better cultural acceptability and scarcer side effects. Roots , stems ,Leaves, flowers, fruit, bark and seeds can all be components of herbal medicines The therapeutic values of medicinal plants lie in possessing phytochemical components which produce certain positive physiological actions on the body of human (Mohammed et al., 2013).

Moringa species are completely documented medicinal tree due to their extraordinary therapeutic and nutritional properties. *Moringa oleifera*, It is one of 14 species belongs to *Moringa*

genus are the most widely cultivated species of the Moringaceae family (Fahey, 2005).

Pharmacological studies on *Moringa oleifera* has begun as early in 1950s which led to the discovery of antibacterial activity in the plant.1 recent study helped to authenticate many of *M. oleifera's* traditional claims such as anticancer and antibacterial properties (Kalappurayil and joseph, 2017). They have long been documented in society medicine as entertaining value in treating a broad variety of ailments. They have been recorded to be antibiotic, detoxifiers, anti-helminthic, immune enhancers and have been used for treatment of malaria disease (Thilzaet al., 2010). The "Moringa" tree is considered one of the worldwide useful trees, fortunately every part of this tree can be used for food or has other advantageous properties. It is used as forage for livestock, In the tropics it is used as a micronutrient powder to treat various complaints in many countries (Devendra et al., 2011).Many reports on disease prevention by using *M*orenga *oleifera* have been reported. The leaves extract is having the ability of diminishing dyslipidemia and hyperglycemia (Mbikay, 2012).Also (Sathya et al., 2010) reported that leaves ethanolic extract prevented DNA damage and cyclophosphamide-induced micronucleus formation in experimental mice .additionally, it has been reported that the leaves extract had strong antiproliferative prompt and apoptotic inducing factor against cancer (KB) cell line (Sreelatha et al., 2011), and it also decreased the cytotoxicity of chemotherapy on pancreatic tumor cells (Berkovich *et al*., 2013).

Up to present time, a diversity of biological activities from different *Morenga oleifera* parts have been documented. However, there are inadequate evidences for *Morenga oleifera* leaf in terms of tumor prevention and therapy. Therefore, the aim of the present paper was to investigate in vitro antiproliferative effect of *Morenga oleifera* leaves methanolic extract against two types of cancer cell linesLB20(female intestine cells of Albino mice)and MCF-7(human breast cancer), furthermore, to identify biologically active chemical compounds in plant extract using GC Mass assay.

MATERIALS AND METHODS

Plant material

Moringa oleifera was cultivated by the researchers(during February 2018) , authenticated by a plant taxonomists in Department of biology/ Mustansiriyah university/Iraq then the leaves collected during November 2018 to prepare alcoholic extract that used for detection the cytotoxicity properties.

Figure(1): *Morenga oleifera* after cultivation (taken by researchers)

Preparation of plant extracts

Plant leaves have been dried at 37^0C, powdered and extracted in alcohol (methanol) by

maceration using procedure adopted by (Priadarshini et al., 2013). Briefly, five gm of leaves powder was dissolved with 100 ml of methanol alcohol in a conical flask and regularly shaken for six hours, then kept undisturbed for the next 18 hours. The macerate thus obtained was filtered with Whatman No. 1 filter paper and filtrate was collected in clean beaker and allowed to dry at oven 400C. The obtained filtrate was weighted and kept at −80 C in the dark till used for further analysis.

In Vitro Anticancer activity

For detection of anticancer activity for methanolic extract from *Morenga oleifera* against MCF7and L20B cell lines that we obtained from Biotechnology research center in Al-Nahrain University. The method of MTT (colorimetric cell viability) was applied as demonstrated by (Chih,et al., 2004)and (Freshney, 2012) since the cells at concentration of 106 cell/ml were cultured in 96-well tissue culture plate. Serial concentrations of methanolic extract ranged at 12.5, 6.25, 3.125 and 1.56mg/ml were prepared by using distilled water. After that 100 µl of each prepared concentration was added to each well and incubated at 37◦C for 24h and 28h, respectively. 10µl of MTT solution (5 mg/ ml) was added to each well then the plate incubated for 4 h at 37◦C. After the limited time 50 µl of DMSO (dimethyl sulfoxide) was added to each well and incubated for 10 min. Negative control was prepared by using normal cell line (REF) as described above. The absorbency was determined by ELISA reader at 620 nm for each used well. The percentage of inhibition was determined with application the following formula:

$$GI\% = \frac{(OD.of\ control\ wells - OD.of\ test\ wells)}{OD.\ of\ control\ wells} \times 100$$

The anticancer efficacy of methanolic extract from *Morenga oleifera* leaves was evaluated against two cell lines (MCF7and L20B) which were provided by the center Biotechnology Research Center of Al-Nahraine University. The colorimetric cell viability MTT assay was used as described by (Chih, et al., 2004) and (Freshney, 2012) (106 cell/m) were cultured in 96-well tissue culture plate. Different concentrations of plant methanolic extract test solution were prepared to evaluate cytotoxic effect against two examined cell lines (12.5, 6.25, 3.125 and 1.56mg/ ml) in water. Then, 100 µl of prepared concentrations was added to each well and incubated for 24h and 28h at 37◦C.After the incubation, a volume of MTT solution (10µl) with concentration (5 mg/ ml) was added to each well and incubated for 4 h at 37◦C.Finally, 50 µl of DMSO (dimethyl sulfoxide) was added to each well and incubated for 10 min. Normal cell line (REF) was added to well (control negative). Then we measured the absorbance for each well using an ELISA reader at 620 nm .Only viable cells able to take the stain while the dead cells were not. The percentage of viability and inhibition ratio of live cells, were calculated according to the formula

$$GI\% = \frac{(OD.of\ control\ wells - OD.of\ test\ wells)}{OD.\ of\ control\ wells} \times 100$$

Identification of chemical constituents of extract using Gas Chromatography-Mass Spectrometry

For GC-MS analysis, a high-temperature column was purchased from Agilent Technologies (SHIMADZU—Japan), this column characterized with (Inert cap 1MS; 30 m × 0.25 mm id × 0.25µm thickness of film), by employing a high-temperature column. Derivatization of every sample was eradicated. The injector and detector temperatures were set at 280°C while the initial column temperature was set at100°C. About 5 µL volume of sample was injected into the column then ran using split (1:10)mode After 1 min, the temperature of oven was raised to 225°C at a rate of ramp 12.5°C/min(hold time 4 min). The oven temperature was then elevated to 300°C at a rate of ramp 7.5°C/min (hold time 5 min).The helium carrier gas was programmed to maintain a constant flow rate of 17.5mL/min and the mass spectra were acquired and processed using both Solution (SHIMADZU—Japan) , Agilent GC-Mass and post run software. The compounds of plant extract have recognized by comparison of their mass with NIST library search and authentic standards.

RESULTS AND DISCUSSION

Cell line growth and cytotoxicity assay

During this study, human breast cancer cell line (MCF7) and the human cancer cell line L20Bwere used as in vitro model to measure anticancer activity of hot methanolic extracts of *Morenga oleifera* leaves. It was found that leaves extracts showed potential cytotoxic effect against both tested cell lines as compared to the cell control.

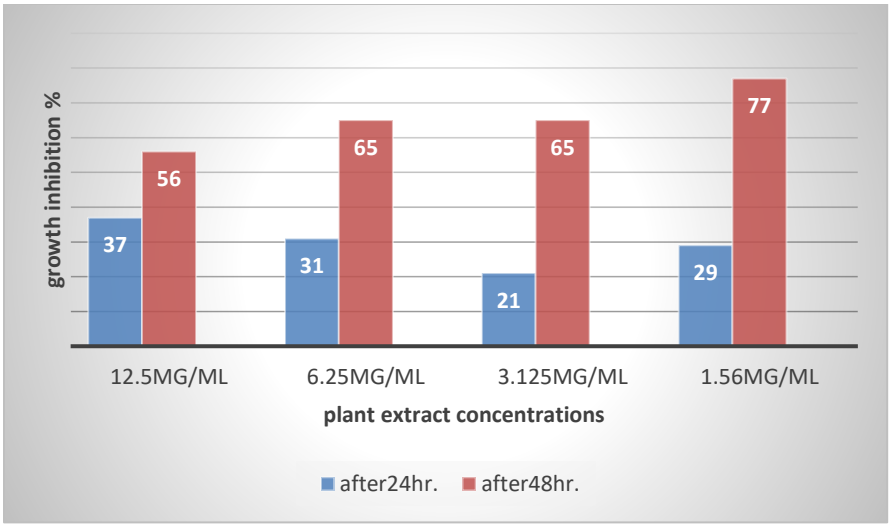
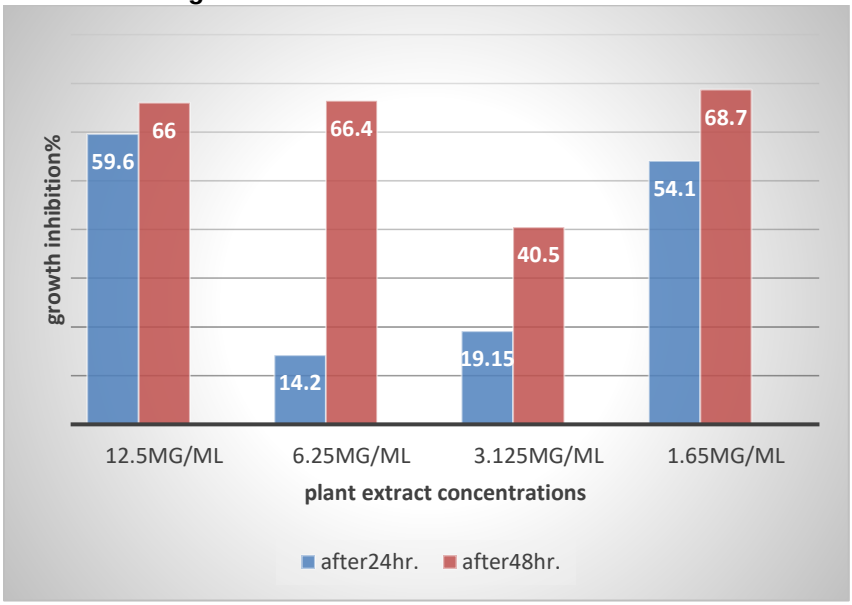


Figure (2): growth inhibition percentage of different concentrations of *Morenga oleifera* leaves methanolic extract on growth of MCF7cell line after 24 and 48 hr. incubation time.



Figure(3): growth inhibition percentage of different concentrations of *Morenga oleifera* leaves methanolic extract on growth of L20B cell line after 24 and 48 hr. incubation time.

Since four different concentrations (12.5, 6.25, 3.125 and 1.56 mg/ml) of methanol extract were applied and the percentage of growth inhibition was calculated after 24 and 48 hr. from incubation. According to the results the maximum percentage of growth inhibition (37%) and (59.6%) was observed with the treatment using 12.5 mg/ml against MCF7 and L20B, respectively. These percentages of inhibition were increased after 48 hr. of incubation to (56%) against MCF7 cell line and (66%) against L20B cell line, While the minimum growth inhibition percentage was calculated as (21%) with the treatment using 3.125 mg/mL and (14.2%) with the treatment using(6.25) mg/mL against MCF7 cell line and L20B cell line, respectively, also these percentages were increased after 48 hr. application to (65%) againstMCF7 cell line, and (66.4%) against L20B cell line. (Fig.2&3)

Naturally occurring anti-tumor constituents from medicinal plants, especially those with high potency and low toxicity, have important suggestions for chemotherapy and chemoprevention. (Li et al.,2010).Among these plants, edible *M. oleifera* is authenticated to be a rich source of various compounds with highly nutritive value and has therefore been regarded as an essential crop (Dahot et al.,1985).

Several researchers have studied the effect of different parts of *Morenga oleifera* against different human cell lines. Since the findings of Shaban et al., (2012) revealed that seed methanolic extract of *Morenga oleifera* showed 80% growth of inhibition against lung (A-549) cell line and in case of liver human cancer line (HT-29) and neuroblastima (IMR-32) cell line plant seed extract showed 95% and 93% activity respectively. Also Charoensin (2014) reported that methanolic and dichloromethane leaves extract from *Morenga oleifera* exhibited strong anti-proliferative effect against breast adenocarcinoma (MCF-7), colorectal adenocarcinoma (Caco-2) and Human hepatocellular carcinoma (HepG2). Another research (Patel et al.,2018)reported that cold methanolic extraction of powdered leaves from *Morenga oleifera* showed anti-cancer effect on various human cancerious cells like DU-145(Prostatecancer), MCF-7(Breast cancer), HEP-3B (liver cancer), K-562 (Leukemia) and HCT-15(colon cancer).

Many authors' studies explained the exact mechanisms for *Morenga oleifera* anticancer activity. Since the findings of Wagdy et al.,(2014) suggested that the role of *Moringa oleifera* leaf extract in decreasing the activity of tumor through down-regulation the expression of apoptosis associated genes(p53 mutation ,p21 and bcl2) as well as through increasing the activity of antioxidant enzymes and/or regulation the intracellular calcium. In addition, *Moringa oleifera* leaf extract have the ability to diminish the induced DNA damage acting as bio-antimutagenic agents.

Tiloke et al.,(2013) Reported that *Moringa oleifera* exhibits potential anti-cancer activity by interfering with the signal transduction cascade that encourages proliferation and development of tumor cells.

Breast cancer is the most widely diagnosed and leading to mortality among women worldwide, traditional medication practices employ plants in prevention, dealing or management of this serious disease (Onyancha et al., 2018). Patients suffering from cancer use herbal medicines to complement or as alternatives for conventional treatment(Njoroge and Kibunga, 2007).Strong ant proliferative effects against breast cancer cell line in current study explained previously by Al-Asmari et al.,(2015),the mentioned author noticed detectable dose dependent increasing in total numbers of apoptotic cells, decreasing in cell motility and colony formation, low cell survival and G2/M enrichment when the breast cancers cells were treated with *Moringa oleifera* leaf and bark extracts .

in the data of present study, *Morenga oleifera* leaves extracts can provide a cheap and sustainable medicine toward breast cancer and can ultimately improve the life quality of the countryside and peri-urban people in developing countries.in addition, The anticancer activity of crude methanolic extract from this plant leaves against L20B cell line is being reported for the first time in the current study.

Identification of chemical constituents of extract usingGas Chromatography-Mass Spectrometry

Fifteen chemical compounds were identified from leaves methanolic extract of *Morenga oleifera*. These compounds with their retention time, chemical formula and composition area percentage are presented in table (1), figure (4)

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Table (1). Major Phyto-components obtained through the GC/MS Study of *Morenga oleifera* leaves extract leaves extract

NO. of peak	Compound name	Chemical formula	Retention time	Composition Area%
1	Acetic acid, 2-methylpropyl este	C6H12O2	3.360	3.78
2	Hexadecanoic acid	C16H32O2	14.835	0.89
3	Tetradecanoic acid	C14H28O2	17.210	0.90
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	18.083	2.09
5	Pentadecanal	:C15H30O	18.550	0.84
6	Tetradecanoic acid	C14H28O2	19.423	14.41
7	Hexadecatrienal	:C16H26O	21.286	55.04
8	Cyclopropanebutanoic acid	C25H42O2	21.443	3.45
9	pentadecen-1-ol	:C16H32O	22.161	0.77
10	Eicosanoic acid	C20H40O2	22.588	1.50
11	14-Methyl-8-hexadecyn-1-ol	:C17H32O	23.731	4.78
12	Oleyl Alcohol	C18H36O	24.152	6.16
13	Cyclooctene	C12H18	24.303	0.91
14	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	:C21H42O4	24.466	1.97
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	26.307	2.53

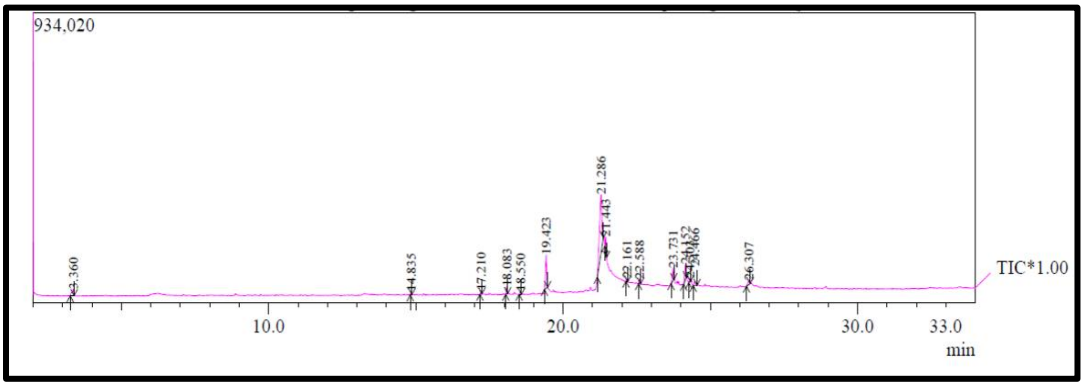

Figure (4): GC-Mass spectrophotometery chromatogram presented the methanolic extract of *Morenga oleifera* leaves

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CONCLUSION

During this study, we could provide the presence of in vitro cytotoxicity effects of *Morenga oleifera* leaves extract against L20B and MCF7 cell lines besides to identification of many tumor preventative constituents by GC Mass analysis that give promising for using this plant as alternative drug to treat cancer in the future. Further investigations for its use in the case of especially clinical breast cancer therapy are needed.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

A.N.M performleed the experiments of anticancer activity., R.J.F designed and performleed the experiments and also wrote the manuscript and data analysis, A.K.A Plant cultivation, collecting plant leaves and extracting them, A.S.D designed and performleed the experiments and also wrote the manuscript and data analysis. All authors read and approved the final version.

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