Cytotoxicity of Cryptochlorogenic acid against Breast cancer cell line (MCF7) isolated from *Moringa oleifera* Leaves Cultivated in Iraq

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Abstract:

M. oleifera L., a Moringaceae family is fast-growing tree. M. oleifera's dried leaves are Characterized with high in phenolic compounds. phenolic compounds have anti-cancer, anti-microbial, anti-inflammatory, anti-allergic and antioxidant activities.

Therefore, The current study involved isolation Cryptochlorogenic acid by preparative TLC from M.oleifera leaves grown in Iraq, and study its cytotoxicity effect against a breast cancer cell line (MCF7) using the MTT cell viability assay, and comparing it to a standard anticancer drug (Tamoxifen). The isolated Cryptochlorogenic acid was cytotoxic to the MCF7 cell line, with an IC50 of 20.8M.

Key words: *Moringa oleifera*, Cryptochlorogenic acid, cytotoxicity against (MCF7).

السمية الخلوية لحمض Cryptochlorogenic ضد خلايا سرطان الثدي (MCF7) المعزول من أوراق المورينجا أوليفيرا المزروعة في العراق

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الخلاصة:

M. oleifera L. ،لعائلة Moringaceae هي شجرة سريعة النمو. تتميز أوراق M. oleifera المجففة باحتوائها على نسبة عالية من المركبات الفينولية. المركبات الفينولية لها أنشطة مضادة للسرطان ، ومضادة للميكروبات ، ومضادة للالتهابات ، ومضادة للحساسية ومضادة للأكسدة. لذلك اشتملت الدراسة الحالية على عزل حامض الكريبتوكلوروجينيك بواسطة TLC التحضيري من أوراق M.oleifera المزروعة في العراق ، ودراسة تأثيره على السمية الخلوية ضد خط خلايا سرطان الثدي (MCF7) باستخدام اختبار قابلية بقاء الخلية MTT ، ومقارنته بعقار معياري مضاد للسرطان (تاموكسيفين). . كان حمض Cryptochlorogenic المعزول سامًا للخلايا لخط خلية MCF7 ، مع IC 50 من M20.8.

الكلمات المفتاحية: البان الزيتي، حمض الكريبتوكلوروجينيك، السمية الخلوية ضد (MCF7).

Introduction

Cancer is one of the leading causes of death worldwide, causing the deaths of nearly seven million people each year [1]. Breast cancer is the leading cause of cancer-related deaths among women in developed countries, as well as the leading cause of all cancer-related deaths globally [2]. Breast cancer has become a major threat to women in Iraq, and it is the most

common type of cancer, accounting for 34.4 percent of the country's most common cancers [3]. Multiple temporal and physiologic changes in cells lead to malignant tumors, making cancer a complex disease [4]. Food additives, non-ionizing electromagnetic exposure, and stress have all been identified as cancer risk factors, with low fruit and vegetable consumption and obesity being the least well-known. [5].

The term "alternative medicine" has gained popularity in Western culture in recent years, and it refers to the practice of using plants for therapeutic purposes. Medicinal plants are widely used as raw materials for extracting active components, which are then used in the production of pharmaceuticals [6].

Moringa oleifera L. is a drought-tolerant, fast-growing, medium-sized perennial tree. It is a member of the Moringaceae family. This tree is common in tropical and subtropical regions around the world, where it has been planted and allowed to spread naturally [7]. It can grow in a wide range of soil types, but prefers well-drained loam to clay loam in neutral to slightly acidic soils. It cannot, however, tolerate standing water for long periods of time. Temperatures between 26 and 40 degrees Celsius are ideal for growth [8]. M. oleifera is native to the Indians and has become naturalized throughout the tropical and subtropical regions of the world. The tree is also known as the Drumstick Tree and the Horseradish Tree [9]. *M. oliefera* is a source of alkaloids, and other triterpenoids as well as flavonoids, tannin, saponin, glycosides and carbohydrates [10]. In addition to high levels of protein and minerals, M. oleifera leaves as seen in figure [1] contain all nine essential amino acids as well as vitamins A, B, and C [11]. It was found that the main active components of *M.* oleifera leaf extracts were cryptochlorogenic acid, astragalin, and isoquercetin [12]. Cryptochlorogenic acid (CCGA) is a phenylpropanoid compound derived from caffeoylquinic acid that has anti-inflammatory and antioxidant properties [13].

Figure (1): Iraqi leaves of M.oleifera

Aim of this study

isolation of Cryptochlorogenic acid from M.oleifera leaves grown in Iraq, and study its cytotoxicity effect against a breast cancer cell line (MCF7).

Materials and method

Plant collection:

M. oleifera mature leaves were collected from Al-Qarieat in Baghdad in October 2020. Classification of the samples was done by Sukaina Abbas from the University of Baghdad's biology department. The leaves were washed in water and then dried. The dried leaves were ground into powder and sealed in containers until needed.

Extraction:

Separately, 100 g of dried powdered leaves were defatted in a thimble with 850 mL of hexane. In a soxhlet extractor, 70 percent ethanol (850 mL) was used to extract the defatted plant material [14]. The extraction was filtered using Whatman No. 1 filter paper. Under reduced pressure, the ethanolic extract was evaporated and then 35 ml of distilled water was added to the concentrated filtrate. [15]. After partitioning with petroleum ether and ethyl acetate, the extraction was partitioned three times with 150mL of n-butanol. The Identification and detection of cryptochlorogenic acid was done by HPLC and TLC.

Isolation and purification of Cryptochlorogenic acid by PLC:

Two grams of n-butanol fraction were subjected to preparative TLC in a solvent system containing ethyl acetate, formic acid, and H2O. (80:10:10) [16]. Bands were identified at 254nm and 366nm using UV light. purity of isolated compound was checked using analytical TLC Comparing with to the standard.

Cytotoxicity Assay:

Cryptochlorogenic acid from Iraqi M. oleifera was examined on human carcinoma cell lines (MCF-7) using the MTT assay to study its effect on cell viability. Following cell culture, trypsinization, and resuscitation, 100 µl of cell suspensions (MCF-7) were dispensed into 96-well plates (flat-bottom tissue culture dishes) and incubated for 24 hours under standard conditions. MTT was used to determine cell viability. An isolated compound extracted from M. oleifera leaves extract was subjected to cells at concentrations ranging from 1.56 µM to 100 µM over a period of 24 h, 48 h, and 72 h Approximately, four hours at 37 C, 30 µl of MTT (contains 3 mg/ml in PBS) was added to the cells, which were then gently tapped onto paper and the medium was gently inverted. To avoid DMSO crystallization, each well on the plates received 100 µl of the solvent at room temperature and in complete darkness. It was possible to measure the absorbance at 540 nm, which is the same wavelength as the standard reference, by means of a microplate reader [17]. Cell absorption into treated versus control cells was used to determine cytotoxicity of the isolated compound [18].

Results and Discussion

HPLC analysis for phenolic acid in n-butanol fraction revealed the presence of cryptochlorogenic acid in the leaves of Moringa plant. Cryptochlorogenic acid was determined by comparison of the retention time (Rt) of compounds in n-butanol extract (5.978) to retention time of cryptochlorogenic acid standard (6.484) as shown in figure (2) and (3) respectively.

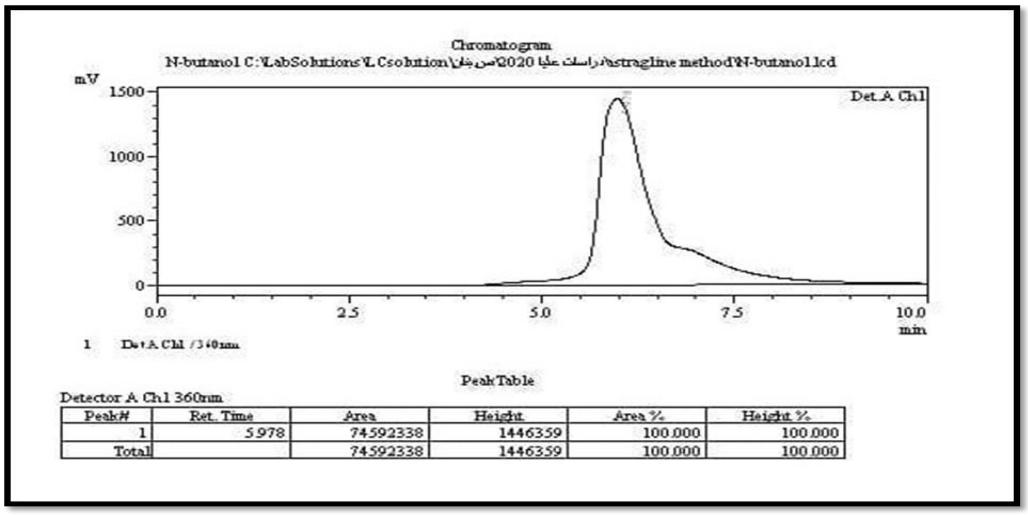

Figure. (2): HPLC chromatogram for n-butanol fraction

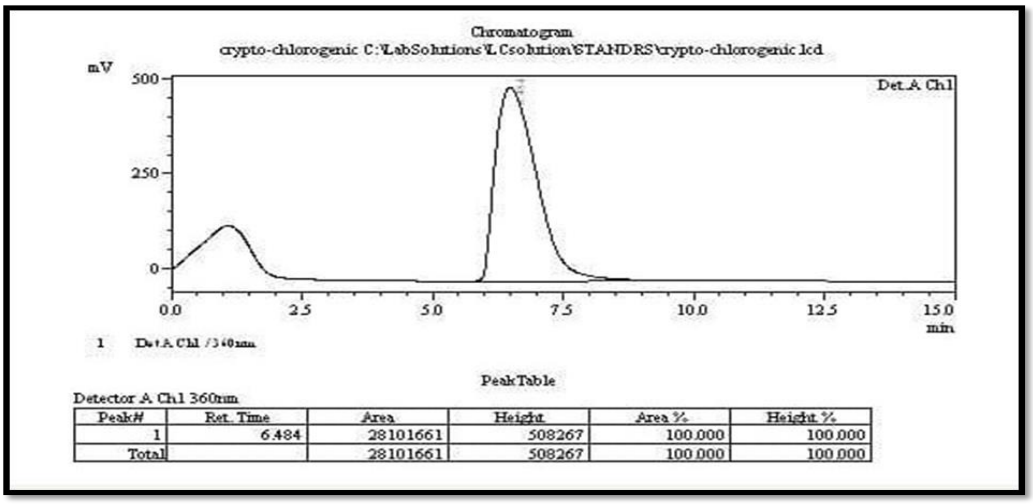

Figure. (3): HPLC chromatogram for cryptochlorogenic acid standared

cryptochlorogenic acid is reported to be present in the extract of M.oleifera. Identification of cryptochlorogenic acid in n-butanol fraction by analytical TLC done with three solvent system as seen in figure (4) and table (1) in comparison with cryptochlorogenic acid standard.

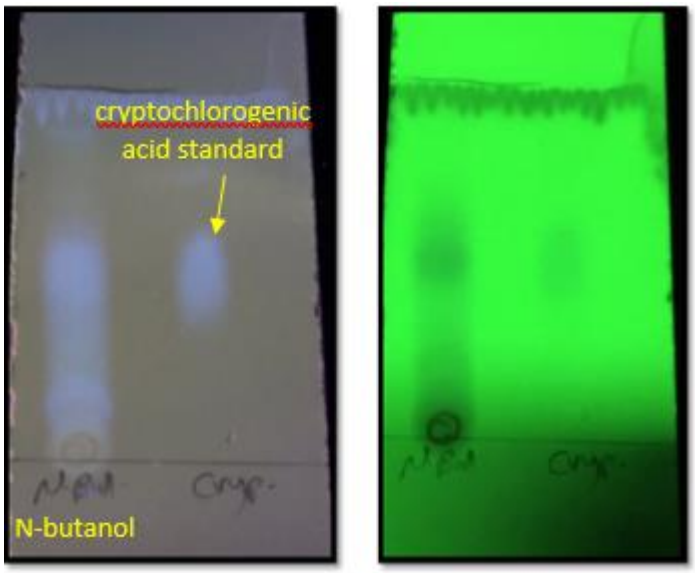

Figure (4): Analytical TLC of standard cryptochlorogenic acid and n-butanol extract under using S5 Solvent system A: at 365nm UV light B: at254 nm UV light

Table (1): Rf values of cryptochlorogenic acid in the n-butanol fraction of ethanolic extract of Moringa leaves and standard cryptochlorogenic acid using S5, S6 and S9 solvent system.

Mobile Phase No.	Solvent system	Rf value of cryptochlorogenic scid standard	Rf value of cryptochlorogenic acid In n-butanol fraction
S5	ethyl acetate: formic acid: H2O (80:10 :10)	0.471	0.471
S6	ethyl acetate: glacial acetic acid: formic acid :H2O (100:11:11:25)	0.633	0.633
S9	ethyl acetate: formic acid: methanol: H2O (20: 0.5: 2.5: 2)	0.571	0.571

Cryptochlorogenic acid was detected under UV light at 365nm and 254nm as seen in Figure (5). The band matching to cryptochlorogenic acid standard was scratched, then eluted with a mixture of acetone and methanol, then the solvent was evaporated at low pressure and temperature. Quantitatively, 120 mg (6) % of the isolated compound was obtained. The isolated compound was evaluated for its cytotoxity against MCF-7 cell line

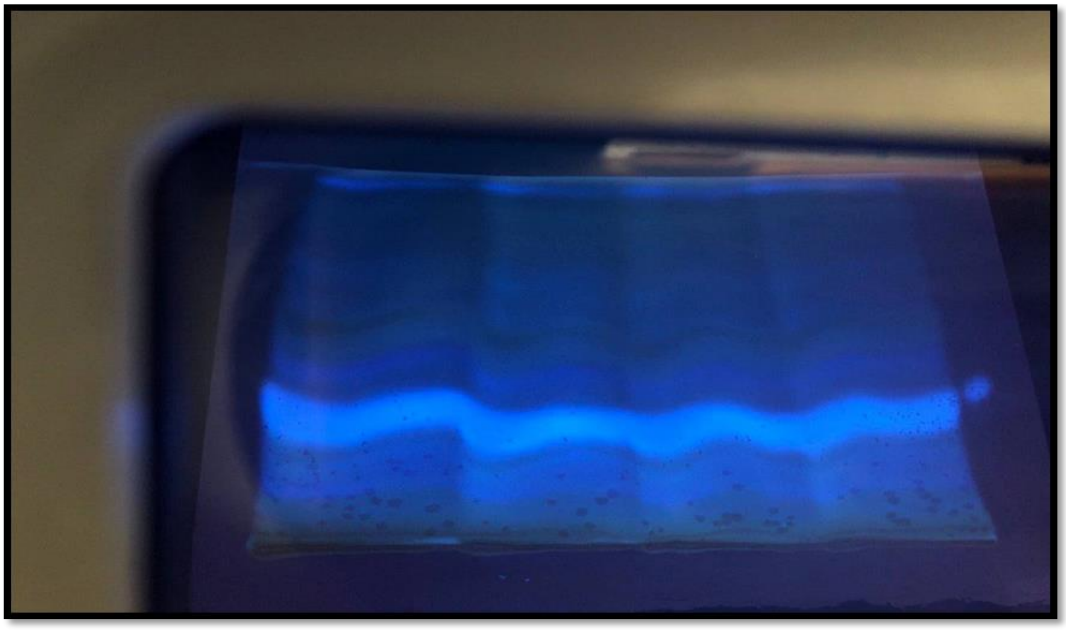

Figure (5): Preparative TLC for isolation and purification of cryptochlorogenic acid at 365nm UV light

In vitro to evaluate the effect of isolated cryptochlorogenic acid extract on human MCF-7 cells death. MCF-7 cells were detected by MTT assay in 96-well plates and treatment with (1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µM) dose ranges of isolated compounds from leaves extract of M. oleifera at 24, 48 and 72 h, then compared with Tamoxifen as control. This study shows that cryptochlorogenic acid caused MCF-7 cells dies, and that the cell death increased with concentration. At 100 µg/ml, it produced 70% cell death after 72 hours, compared to Tamoxifen, which produced 95% as shown in the figure (6), at the same time and concentration. The present study also revealed that Cryptochlorogenic acid have significant effect in the cell death of breast cancer and according to IC50 values which include (20.8 µM) compared to the IC50 for Tamoxifen (Control) in the same cell line (18.02 µM) as shown in figure (7) and (8). The results of Cryptochlorogenic acid in this study were consistent with those of a previous study done by Elansary HO and Szopa A., which found that Cryptochlorogenic acid had high antioxidant and antiproliferative activities against breast adenocarcinoma (MCF7), colon adenocarcinoma (HT-29), and cervical adenocarcinoma (HeLa) cell lines (19). The mechanism by which the cell death of breast cancer occur, may be inhibited MCF-7 breast cancer cells' glucose uptake, and interfere with lactate availability leading to altering their metabolism and survivability, or by modulate estrogen receptors by decreasing in estrogen receptor-related signaling which lead to decrease cellular proliferation and increase apoptosis (20).

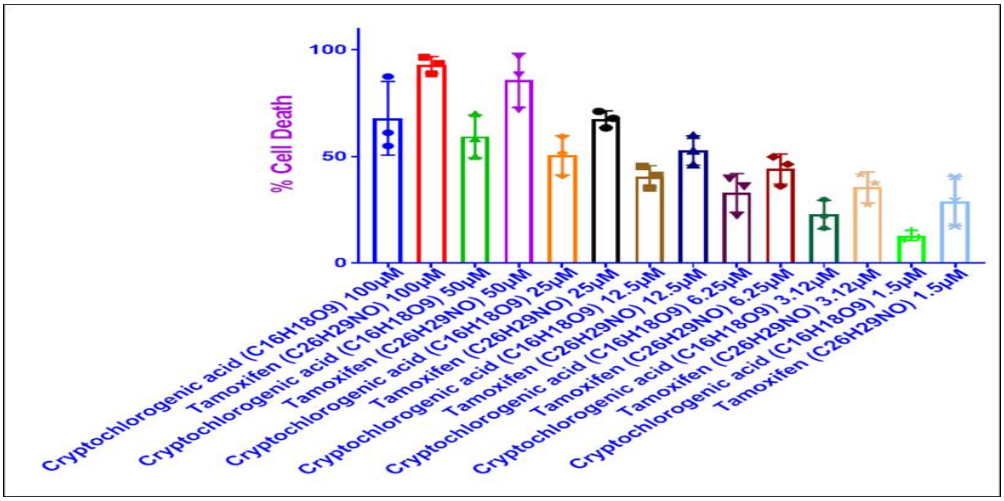

Figure (6): In vitro % cell death of the breast cancer (MCF-7) cells was detected by MTT assay.

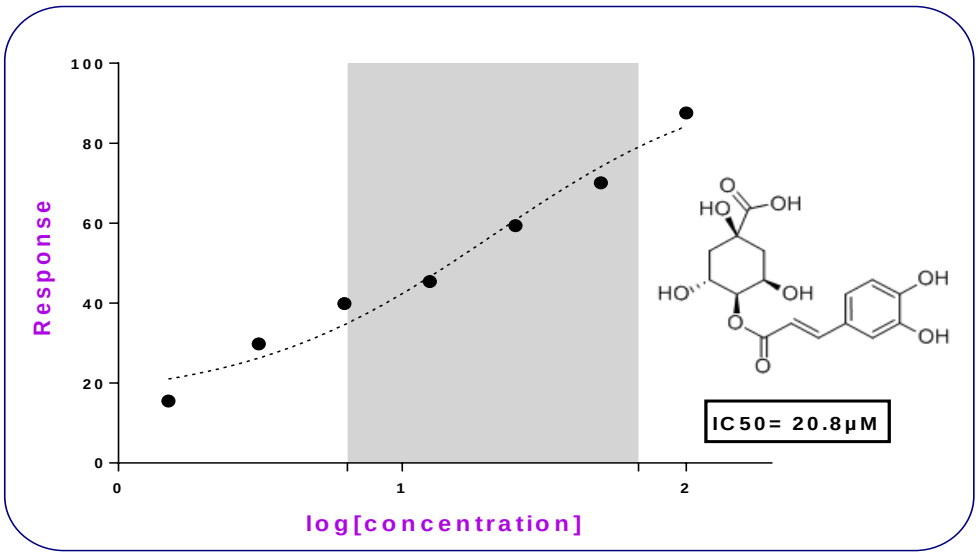

Figure (7): IC50 dose-response curves for Cryptochlorogenic acid (C1). C1 dose ranges of 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 µM were applied to MCF-7 cells for 72 hours. C1 dose response was plotted against log transformed C1 concentrations. Nonlinear regression analysis was used to calculate IC50 values (Prism Pad 8.1).

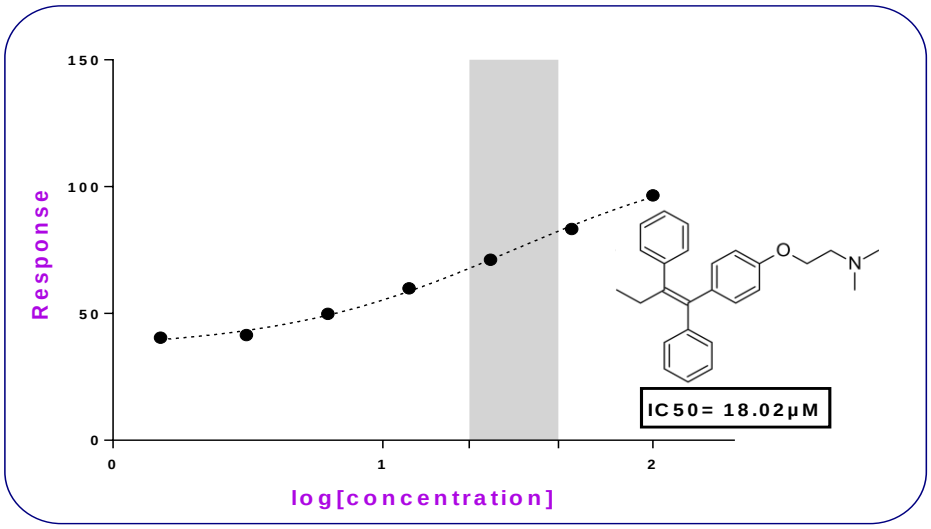

Figure (8): Tamoxifen IC50 dose-response curves (Control). Tamoxifen dose ranges of 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 µM were applied to MCF-7 cells for 72 hours. Tamoxifen dose response was plotted against log transformed Tamoxifen concentrations. Nonlinear regression analysis was used to calculate IC50 values (Prism Pad 8.1).

Conclusion

M.oleifera leaf extract contains cryptochlorogenic acid, a phenolic acid. This compound has a significant effect on the cell death of (MCF-7) cells, according to the IC50 value. Detailed research is needed to fully understand this compound's mechanism of action and its specific molecular target in terms of apoptosis.

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