

Article

In Vitro Evaluation Of Curcumin and Its Analogues (1-6) Against The MDA MB-231 Breast Cancer Cell Lines

Imad khalaf Hassan¹, Rita S. Elis², Shaker A.N Al-Jadaan³

^{1,2,3} Department Of Pharmaceutical Chemistry, Collage Of Pharmacy, University Of Basrah, Basrah, Iraq

* Correspondence: imadkhassan@gmail.com

Abstract: Our previous work included the synthesis and identification of curcumin and its analogues (1-6). This study involved in vitro evaluation of curcumin and its analogues (1-6) as anti-breast cancer against MDA-MB 231 cell line. Curcumin and all analogues (1-6) were assayed in vitro as anti-proliferation against the MDA-MB-231 breast cancer cell line by using the Micro-Culture Tetrazolium (MTT) method. Compound 6 (IC₅₀ 99.36 and SI 2.5) exhibited strong cytotoxic activity and selectivity than curcumin and compounds (1-5). This makes it a more promising selective agent for treating triple-negative breast cancer cell lines (TNBC) than other compounds 1–5.

Keywords: Brest Cancer; Curcumin; MDA-MB-231 cell Line; Selectivity Index; MTT Method.

1. Introduction

Breast cancer is a diverse disease, and one of its types is triple-negative breast cancer (TNBC)(1). It's characterized by the absence of three common types of receptors: estrogen receptor, progesterone receptor, and human growth factor receptor 2 (HER2)(2). Epidermal growth factor receptor 2 (EGFR) is known to trigger the growth of most breast cancer(3). Clinical characteristics common to TNBC include a poor prognosis over the long term or a particular pattern of relapse(4). In comparison to other subtypes of breast cancer, TNBC carries a greater risk of metastasis and a higher death rate(5). Targeted therapy strategies focus on proteins and pathways that were linked to the malignancy, such as (phosphoinositidine-3-kinase)p13K, (protein kinase B) Akt, (mammalian Target of Rapamycin)mTOR, (Transforming Growth Factor Alpha) TGF- α , (Epidermal Growth Factor) EGF, and (Tumor Suppressor Protein) p53, as well as proteins like (Mitogen-Activated Protein Kinase) MAP(6). A single therapeutic strategy is insufficient due to its high cost, lack of safety, and ineffectiveness(7, 8). TNBC, which is classified according to a wide range of different molecular markers and signaling pathways(9). Because of the lack of required receptors, conventional hormone treatment and medications targeting these receptors are ineffective(10). Currently, natural ingredients are used in the production of around half of all medications(11). Numerous plant-derived chemicals with antitumor action for a variety of malignancies have been found in the search for an efficient chemotherapy-preventive regimen(12). Phenolic chemical compounds originating from plants have a wide range of beneficial properties and may be able to stop many stages of carcinogenesis(13, 14). Curcumin, one of the polyphenolic compounds, can inhibit the proliferation of cancer cells, induce apoptosis, stop angiogenesis, control the synthesis of anti-apoptotic proteins, and defend the immune system from tumor carriers, It may have

Citation: Imad khalaf Hassan. In Vitro Evaluation Of Curcumin and Its Analogues (1-6) Against The MDA MB-231 Breast Cancer Cell Lines. Central Asian Journal of Medical and Natural Science 2024, 5(4), 1002-1009

Received: 10th Jul 2024

Revised: 11th Agt 2024

Accepted: 24th Sep 2024

Published: 17th Okt 2024



Copyright: © 2024 by the authors. Submitted for open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>)

surprising therapeutic benefits(15, 16). Pharmacokinetic studies indicate that it has limited systemic bioavailability(17). Curcumin having two aromatic rings, which include hydroxyl and methoxy substitutions, is essential for the binding of the compound to a possible receptor(18). They help stabilize the molecule. It is essential to the anti-proliferative action. Substitution of halogen, methoxy, and hydroxyl significantly increased the compounds' potency(19).

Curcumin analogs that may be less toxic but more effective(20). Therefore, these studies display the effect of curcumin and its analogues in vitro against the MDA-MB-231 breast cancer cell line.

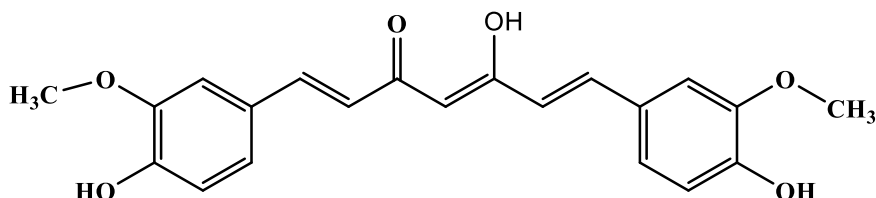


Figure (1). The chemical structure of curcumin.

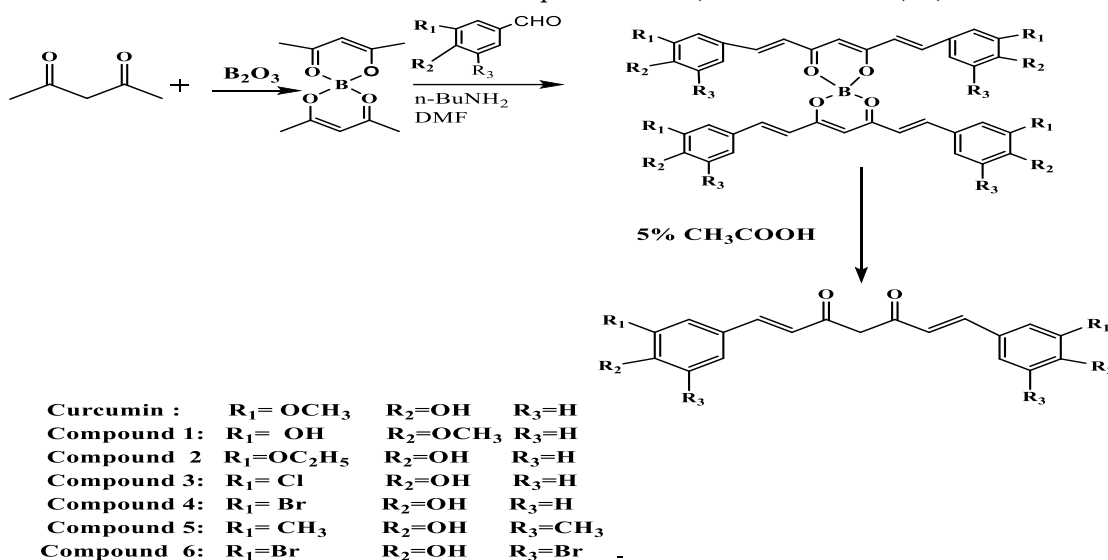
2. Materials and Methods

2.1. Materials

The MDA-MB-231 breast cancer cell line and the HdFn normal cell line were supplied by the Biotechnology Research Center at Al-Nahrain University. The micro culture tetrazolium bromide (MTT) kit was purchased from Intron Biotech Korea.

2.2. The Chemical synthesis of curcumin and its analogues (1-6)

The synthesis and characterization of curcumin and its analogues (compound 1-6) as shown in scheme 1 were mentioned in acceptance letter (AJCA-2409-1671(R1).



Scheme (1). The chemical synthesis of curcumin and its analogues (1-6).

Curcumin (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

From 3-methoxy-4-hydroxybenzaldehyde(7.6g): Yield 7.1g(74.57%), dark yellow powder-IR(KBr disk cm^{-1}):OH str.(3600-3200br,m) Ar-C=C-H(3050w),C-Hstr.(2950w),C=O(1627s),C=Cstr.(1602,1591s)- ^1H NMR(500 MHz-DMSO- d_6): δ =16.34(s,1H), δ 7.52(d,2H) J =16Hz, δ =6.71 (d,2H) J =16Hz, δ 6.01(s,1H), δ 7.28(s,2H), δ 7.11(d,2H) J =8Hz, δ 6.79(d,2H) J =8.5Hz, δ 3.80(s,6H), δ 9.62(s, 2H)- ^{13}C NMR(125MHzDMSO d_6):(C1)101.3,(C8,C8') 149.8,(C9,C9')148.3(C7,C7')116.2,(C10,10')111.8,(C6,6')123.5,(C5,C5') 126.7 ,

(C4,C4')141.1,(C3,C3')121.5,(C2,C2') 183.5,(R1) 56.1-m.p=180-183°C- λ_{max} 425nm-EIMS(M^+ 368.1) .

Compound 1 (1E,6E)-1,7-bis(3-hydroxy-4-methoxyphenyl)hepta-1,6-diene-3,5-dione From 3-hydroxy-4-methoxybenzaldehyde (7.6 g): Yield 7.31g (76.82%) - Bright yellow powder IR (KBr, cm^{-1}) OH str. (3500-3100br,m), Ar C=C-H (3025w), C-H str (2841 w), C=O (1624 s), C=Cstr (1602,1591s) ^1H NMR (500MHz-DMSO- d_6): δ 16.25 (s,1H), δ 7.46 (d,2H) J =16Hz, δ 6.60 (d,2H) J =16Hz, δ 6.06 (s,1H), δ 7.07-7.10 (m,4H), δ 6.92 (d,2H) J =8.5Hz, δ 9.18 (s,2H), δ 3.77 (s,6H) ^{13}C NMR (125MHz-DMSO- d_6): (C1)101.6, (C8,C8') 150.5, (C9,C9') 147.2, (C7,C7') 114.6, (C10,C10') 112.5, (C6,C6') 122.1, (C5,C5') 128.1, (C4,C4') 140.9, (C3,C3') 121.8, (C2,C2') 183.5, (R2) 56.1-m.p=138-141°C- λ_{max} 417nm-EIMS(M^+) 368.2.

Compound 2 (1E,6E)-1,7-bis(3-ethoxy-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione From 3-ethoxy-4-hydroxybenzaldehyde (8.3g): Yield 5.2g (48.95%), Orange yellow powder IR (KBr, cm^{-1}): OH str. (3600-3100br,m), Ar-C=C-H (3050w), C-H str. (2983s,2933s) C=O (1625s) C=Cstr (1600s,1587s,1510s) ^1H NMR (500MHz-DMSO- d_6): δ 7.49 (d,2H) J =16Hz, δ 6.69 (d,2H) J =16Hz, δ 6.00 (s,1H), δ 7.25 (s,2H), δ 7.10 (d,2H) J =8Hz, δ 6.79 (d,2H) J =8Hz, δ 4.04 (q,4H) J =7Hz, 1.31 (t,6H) J =7Hz, δ 9.51 (s,2H) ^{13}C NMR (125MHz-DMSO- d_6): (C1)101.3, (C8,C8') 150.0, (C9,C9') 147.5, (C7,C7') 116.2, (C10,C10') 113.1, (C6,C6') 123.4, (C5,C5') 126.8, (C4,C4') 141.1, (C3,C3') 121.5, (C2,C2') 183.6, (R1) 64.3, 15.1. - m.p=188-191°C- λ_{max} 425 nm-EIMS(M^+ 396.1).

Compound 3 (1E,6E)-1,7-bis(3-chloro-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione From 3-chloro-4-hydroxybenzaldehyde (7.82g): Yield 2.8 g (28.71%) - yellow powder IR (KBr, cm^{-1}): OH str. (3600-2800br,m), Ar-C=C-H (3025w), C=O (1624s), C=Cstr (1598s,1500s) ^1H NMR (500MHz-DMSO- d_6): δ 16.23 (s,1H), δ 7.45-7.72 (m), δ 6.74 (d,2H) J =16Hz, δ 5.97 (s,1H), 7.45-7.72 (m), δ 6.96 (d,2H) J =9Hz, δ 7.45-7.72 (m), δ 10.77 (s,2H) ^{13}C NMR (125MHz-DMSO- d_6): (C1)102.0, (C8,C8') 155.5, (C9,C9') 130.3, (C7,C7') 117.3, (C10,C10') 127.6, (C6,C6') 122.7, (C5,C5') 129.1, (C4,C4') 139.5, (C3,C3') 120.9, (C2,C2') 183.5- m.p=209-212°C- λ_{max} 413 nm-EIMS(M^+ 376.9).

Compound 4 (1E,6E)-1,7-bis(3-bromo-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione From 3-bromo-4-hydroxybenzaldehyde (10.05 g): Yield 7.94 g (65.92%), dark yellow powder IR (KBr, cm^{-1}): OH str. (3600-2800br,m), Ar-C=C-H (3050), C=O (1622s), C=Cstr (1597s,1515s) ^1H NMR (500MHz-DMSO- d_6): δ 16.21 (s,1H), δ 6.93-7.50 (m), δ 6.74 (d,2H) J =16.5Hz, 5.97 (s,1H), δ 7.87 (s,2H), δ 6.93-7.50 (m), δ 10.82 (s,2H) ^{13}C NMR (125MHz-DMSO- d_6): (C1)102.0, (C8,C8') 156.5, (C9,C9') 133.4, (C7,C7') 117.0, (C10,C10') 110.5, (C6,C6') 122.7, (C5,C5') 128.0, (C4,C4') 139.4, (C3,C3') 129.7, (C2,C2') 183.5- m.p=219-212°C- λ_{max} 415 nm-EIMS(M^+ 465.9).

Compound 5 (1E,6E)-1,7-bis(4-hydroxy-3,5-dimethylphenyl)hepta-1,6-diene-3,5-dione

From 3,5-dimethyl-4-hydroxybenzaldehyde (7.5 g): yield 5.1 g (54.14%), dark orange powder IR (KBr, cm^{-1}): OH str. (3600-2800br,m), Ar-C=C-H (3035), C-H str. (2916w), C=O (1622s) C=Cstr (1571,1531s) ^1H NMR (500MHz-DMSO- d_6): δ 7.43 (d,2H) J =16Hz, δ 6.62 (d,2H) J =16, 5.95 (s,1H), δ 7.25 (s,4H), δ 2.14 (s,6H), δ 8.81 (s,2H) ^{13}C NMR (125MHz-DMSO- d_6): (C1)101.4, 56.5, (C8,C8') 156.3, (C9,C9') 121.1, (C7,C7') 121.1, (C10,C10') 125.1, (C6,C6') 125.1, (C5,C5') 129.7, (C4,C4') 141.0, (C3,C3') 126.2, (C2,C2') 184.2, (R1) 19.0, 16.9, (R3) 19.0, 16.9. - m.p=188-190°C- λ_{max} 425 nm-EIMS(M^+ 364.2).

Compound 6 (1E,6E)-1,7-bis(3,5-dibromo-4-hydroxyphenyl)hepta-1,6-diene-3,5-dione

From 3,5-dibromo-4-hydroxybenzaldehyde (13.99 g) Yield: 5.76 g (35.73%), lemon yellow-IR (KBr, cm^{-1}): OH str. (3600-2800), Ar-C=C-H (3050), C=O (1629s), C=C str. (1579s-1477s)- ^1H NMR (500MHz-DMSO- d_6): δ 16.11 (s, 1H), δ 7.44 (d, 2H) J =16Hz, δ 6.87 (d, 2H) J =16.5Hz, δ 5.96 (s, 1H), δ 7.92 (s, 4H), δ 10.38 (s, 2H)- ^{13}C NMR (125MHz-DMSO- d_6): (C1) 102.7, (C8, C8') 152.8 (C9, C9') 132.6, (C7, C7') 132.6, (C10, C10') 112.7, (C6, C6') 112.7, (C5, C5') 124.4, (C4, C4') 138.0, (C3, C3') 129.8, (C2, C2') 183.4-m.p=253.8-254 $^{\circ}\text{C}$ - λ_{max} 411 nm-EIMS (M^{+} 623.8).

2.3. Cell culture and anti-breast cancer cell line cancer assay by MTT method.

2.3.1. Assay in vitro the MTT

The cytotoxic activity of curcumin and its analogues (1-6) was done in the Biotechnology center /Al-Nahrain University against MDA-MB-231 breast cancer cell line, HdFn normal cell line and using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. A flat 96-well plate was seeded with 100 μL of cells each well, or 1×10^6 cells per well, which let the cells growth in an incubator with 5% CO_2 for 24 hours at 37 $^{\circ}$. Following the incubation period, fresh media containing different concentrations of curcumin and its analogues (25, 50, 100, 200, and 400 $\mu\text{g mL}^{-1}$) were added to each well.

The plate needed to be incubated for a further 24 h. A 10 μL solution of MTT was added to each well after incubation. Another four hours were used for incubating the plate. To solubilize the formazan crystals, 100 μL of DMSO solubilization solution was applied after removing the medium and MTT. After the purple formazan was fully dissolved, the optical density at 570 nm was measured using an ELISA microplate (Bio-Rad, USA), and the percentage of inhibition with respect to the vehicle control (untreated cells) was computed. Each cell line's half-maximal inhibitory concentration (IC_{50}) values are calculated after treatments were done in triplicate⁽²¹⁾.

2.3.2. Cell lines maintenance and the cultures.

The breast cancer cell line MDA-MB-231 and (HdFn) normal cell line, were kept in Roswell Park Memorial Institute (RPMI) media supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 20 mM HEPES Z(4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid), 100 U/mL penicillin G, and 100 $\mu\text{g/mL}$ streptomycin was used for maintaining the cells. After being seeded into tissue culture flasks, the cells were cultured at 37 $^{\circ}\text{C}$ for 24 to 48 hours with an extra 5% CO_2 until they achieved approximately 90% monolayer confluency. Gently trypsinize (solution of trypsin) the cells (2-4 mL, 50 mg mL^{-1}) to harvest the cells⁽²²⁾.

2.4. The biological activity of curcumin and its analogues (1-6).

The preliminary analysis of cytotoxic activity and selectivity of curcumin and its analogues (1-6) against MDA-MB-231 breast cancer cell line and HdFn normal cell using the MTT method⁽²³⁾, by which determined the inhibitory concentration IC_{50} and the selectivity index (SI). The selectivity index was calculated the selective capability of curcumin and its analogues (1-6). (SI) means the ratio of the 50% inhibitory concentration of normal cell (IC_{50}) to 50% inhibitory concentration of the cancer cell (IC_{50}) and calculated by using equation

$$\text{SI} = \text{IC}_{50} \text{ of normal cell} / \text{IC}_{50} \text{ of cancer cell} \quad \text{equation 1}$$

When (SI) values higher than 1.0 indicates that the compound has a great anti-cancer property, while when SI much greater than 1.0 Indicates that the compound is highly selective⁽²⁴⁾. The absorbance was measured at wavelength of 575nm using ELISA reader. Statically analysis was performed on optical density data to determine the (IC_{50}).

3. Results

In vitro cytotoxic activity study of curcumin and its analogues (1-6) against MDA-MB-231 breast cancer cell line

The result obtained from the Table (1) and Figures (3-8) show that, compound 6 with IC_{50} 99.36 and SI 2.5 was classified as superior inhibitory effect and selective agents, while

curcumin and the remaining compounds (1-5) with IC_{50} ranged between 112.6- 215.1 $\mu\text{g/mL}$ are classified as moderate cytotoxic agent⁽²⁵⁾. The results revealed that, the type of substitution on the aromatic rings play an important role in the activity and selectivity of compounds against MDA-MB-231 breast cancer cell line⁽²⁶⁾. The different in activity and selectivity may be due to different substitution on aromatic ring as show in compound 1 (IC_{50} 121.6 $\mu\text{g/mL}$ and SI 1.69) and compound 2 (IC_{50} 112.6 $\mu\text{g/mL}$ and SI 1.14), exhibits higher activity and selectivity than curcumin. While compound 4 (IC_{50} 129.5 $\mu\text{g/mL}$ and SI 1.83) and compound 5 (IC_{50} 140.3 $\mu\text{g/mL}$ and SI 1.43) exhibits activity less than curcumin but with higher selectivity. Compound 3 with IC_{50} 215.1 $\mu\text{g/mL}$ and SI = 0.83, exert less activity and selectivity than curcumin. So, when compared with curcumin the results show that, exchanging the position of the 4-OH with 3-OCH₃ groups or replacing the 3-OCH₃ group with the 3-OC₂H₅ group as seen with compounds 1 and 2 respectively, lead to increase activity and selectivity of compounds⁽²⁷⁾. While when 3-OCH₃ was replaced by either Br or by presence two CH₃ groups ortho phenolic OH as seen in compounds 4 and 5 respectively, lead to decrease activity and increase selectivity of compounds⁽²⁸⁾. Compound with the chloride atom instead of methoxy group, give less activity and selectivity than curcumin⁽²⁷⁾.

Table (1). The IC_{50} and SI values of curcumin and its analogues against MDA-MB-231.

Compounds	IC_{50} of MDA	IC_{50} HdFn	S I
curcumin	125.5	130.1	1.03
1	121.6	206.4	1.69
2	112.6	128.4	1.14
3	215.1	178.8	0.83
4	129.5	237.0	1.83
5	140.3	201.2	1.43
6	99.36	252.9	2.5

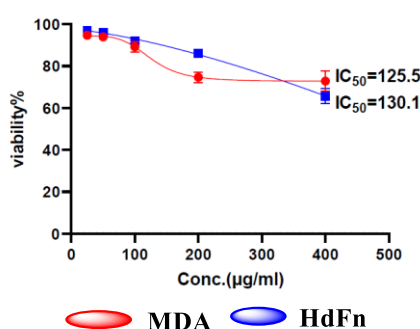


Figure (3). Effect of curcumin on viability

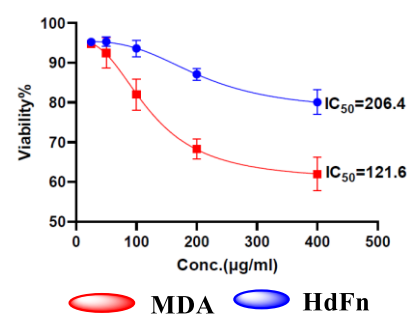


Figure (4). Effect of compound 1 on viability

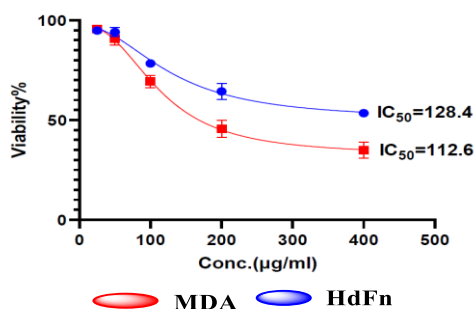
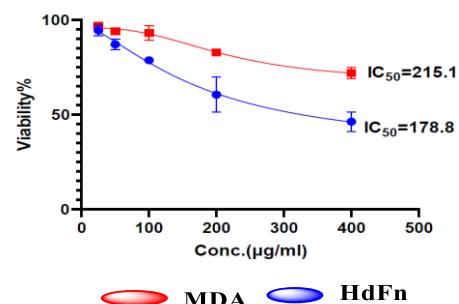


Figure (5). Effect of compound 2 on viability



Figure(6). Effect of compound 3 on viability

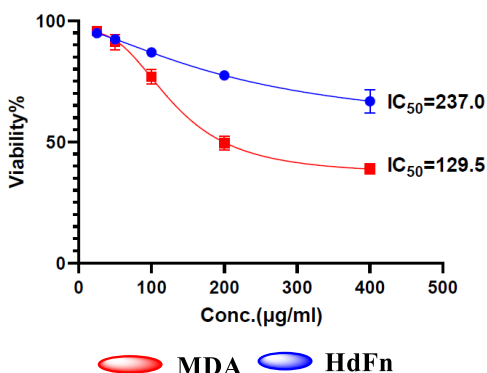


Figure (6). Effect of compound 4 on viability

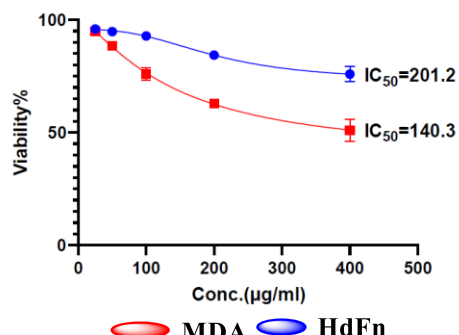


Figure (7). Effect of compound 5 on viability

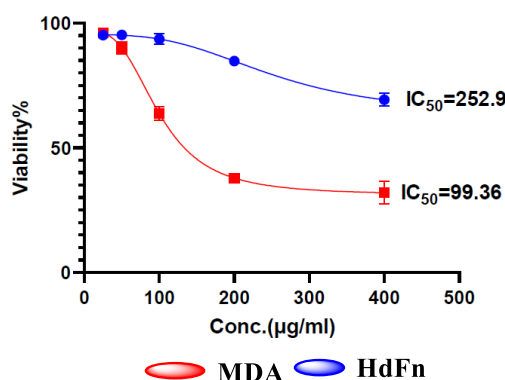


Figure (8). Effect of compound6 on viability

4. Conclusion

On the basis of our results for evaluation of anti-proliferation activity of the synthesized compounds curcumin and its analogues against the MDA-MB-231 breast cancer cell line by using MTT method demonstrated that the activity and selectivity depend on substitution on the aromatic rings. The result revealed compound 6 possesses a stronger inhibitory effect and is more selective towards MDA-MB-231 breast cancer cell line compared to curcumin and compounds 1–5.

REFERENCES

- [1] Kumar P, Aggarwal R. An overview of triple-negative breast cancer. Archives of gynecology and obstetrics. 2016;293:247-69.
- [2] Changavi AA, Shashikala A, Ramji AS. Epidermal growth factor receptor expression in triple negative and nontriple negative breast carcinomas. Journal of laboratory physicians. 2015;7(02):079-83.

- [3] Shilpi S, Shivvedi R, Khatri K. Triple Negative Breast Cancer (TNBC): A challenge for current cancer therapy. *J Hum Virol Retrovirol*. 2018;6:00189.
- [4] Qiu J, Xue X, Hu C, Xu H, Kou D, Li R, et al. Comparison of clinicopathological features and prognosis in triple-negative and non-triple negative breast cancer. *Journal of cancer*. 2016;7(2):167.
- [5] Ocana A, Pandiella A. Targeting oncogenic vulnerabilities in triple negative breast cancer: biological bases and ongoing clinical studies. *Oncotarget*. 2017;8(13):22218.
- [6] Kirouac DC, Du J, Lahdenranta J, Onsum MD, Nielsen UB, Schoeberl B, et al. HER2+ cancer cell dependence on PI3K vs. MAPK signaling axes is determined by expression of EGFR, ERBB3 and CDKN1B. *PLoS computational biology*. 2016;12(4):e1004827.
- [7] Thakur V, Kutty RV. Recent advances in nanotheranostics for triple negative breast cancer treatment. *Journal of Experimental & Clinical Cancer Research*. 2019;38(1):430.
- [8] Alsaad H, Kubba A, Tahtamouni LH, Hamzah AH. Synthesis, docking study, and structure activity relationship of novel anti-tumor 1, 2, 4 triazole derivatives incorporating 2-(2, 3-dimethyl aminobenzoic acid) moiety. *Pharmacia*. 2022;69:415-28.
- [9] Segovia-Mendoza M, Romero-Garcia S, Lemini C, Prado-Garcia H. Determining Factors in the Therapeutic Success of Checkpoint Immunotherapies against PD-L1 in Breast Cancer: A Focus on Epithelial-Mesenchymal Transition Activation. *Journal of Immunology Research*. 2021;2021(1):6668573.
- [10] Zhao L, Zhou S, Gustafsson J-Å. Nuclear receptors: recent drug discovery for cancer therapies. *Endocrine reviews*. 2019;40(5):1207-49.
- [11] Pan S-Y, Zhou S-F, Gao S-H, Yu Z-L, Zhang S-F, Tang M-K, et al. New perspectives on how to discover drugs from herbal medicines: CAM' S outstanding contribution to modern therapeutics. *Evidence-Based Complementary and Alternative Medicine*. 2013;2013(1):627375.
- [12] Kapinova A, Stefanicka P, Kubatka P, Zubor P, Uramova S, Kello M, et al. Are plant-based functional foods better choice against cancer than single phytochemicals? A critical review of current breast cancer research. *Biomedicine & Pharmacotherapy*. 2017;96:1465-77.
- [13] Kapinova A, Kubatka P, Golubnitschaja O, Kello M, Zubor P, Solar P, et al. Dietary phytochemicals in breast cancer research: anticancer effects and potential utility for effective chemoprevention. *Environmental health and preventive medicine*. 2018;23:1-18.
- [14] Badran HA, Al-Maliki A, Alfahed RF, Saeed BA, Al-Ahmad A, Al-Saymari F, et al. Synthesis, surface profile, nonlinear reflective index and photophysical properties of curcumin compound. *Journal of Materials Science: Materials in Electronics*. 2018;29:10890-903.
- [15] Huang Y, Jiang X, Liang X, Jiang G. Molecular and cellular mechanisms of castration resistant prostate cancer. *Oncology letters*. 2018;15(5):6063-76.
- [16] Abd. Wahab NA, H. Lajis N, Abas F, Othman I, Naidu R. Mechanism of anti-cancer activity of curcumin on androgen-dependent and androgen-independent prostate cancer. *Nutrients*. 2020;12(3):679.
- [17] Phansalkar PS, Zhang Z, Verenich S, Gerk PM. Pharmacokinetics and bioavailability enhancement of natural products. *Natural Products for Cancer Chemoprevention: Single Compounds and Combinations*. 2020:109-41.
- [18] Chainoglou E, Hadjipavlou-Litina D. Curcumin in health and diseases: Alzheimer's disease and curcumin analogues, derivatives, and hybrids. *International journal of molecular sciences*. 2020;21(6):1975.
- [19] Sethi S, Choudhary S, Sharma D, Jyothi VGS, Baldi A, Madan NKMJ. Armamentarium of anticancer analogues of curcumin: Portray of structural insight, bioavailability, drug-target interaction and therapeutic efficacy. *Journal of Molecular Structure*. 2021;1231:129691.
- [20] Panda SS, Girgis AS, Thomas SJ, Capito JE, George RF, Salman A, et al. Synthesis, pharmacological profile and 2D-QSAR studies of curcumin-amino acid conjugates as potential drug candidates. *European Journal of Medicinal Chemistry*. 2020;196:112293.
- [21] Jaafar ND, Al-Saffar AZ, Yousif EA. Genotoxic and cytotoxic activities of lantadene A-loaded gold nanoparticles (LA-AuNPS) in MCF-7 cell line: an in vitro assessment. *International journal of toxicology*. 2020;39(5):422-32.
- [22] Mahmood RI, Abbass AK, Al-Saffar AZ, Al-Obaidi JR. An in vitro cytotoxicity of a novel pH-Sensitive lectin loaded-cockle shell-derived calcium carbonate nanoparticles against MCF-7 breast tumour cell. *Journal of Drug Delivery Science and Technology*. 2021;61:102230.
- [23] Ali Z. Al-Saffar, Fatimah A. Sabry, Shymaa L. Al-Brazanchi, Ahmed F. Al-Shanon, Firas Hassan and Noora A. Hadi. Phytochemical analysis, antioxidant and cytotoxic potentials of Pelargonium graveolens extract in human breast adenocarcinoma (MCF-7) cell line *Asian J Biochem*. 2017;12:16-26.

-
- [24] Jihad RS, Abdul-Rida NA, Al-Shamari AM, Al-Masoudi NA, Saeed BA. Design, synthesis, and in-silico study of new letrozole derivatives as prospective anticancer and antioxidant agents. *Zeitschrift für Naturforschung B*. 2023;78(6):343-53.
- [25] Weerapreeyakul N, Nonpunya A, Barusrux S, Thitimetharoch T, Sripanidkulchai B. Evaluation of the anticancer potential of six herbs against a hepatoma cell line. *Chinese medicine*. 2012;7:1-7.
- [26] Bastos PM, Eriksson J, Green N, Bergman Å. A standardized method for assessment of oxidative transformations of brominated phenols in water. *Chemosphere*. 2008;70(7):1196-202.
- [27] Zhao C, Liu Z, Liang G. Promising curcumin-based drug design: mono-carbonyl analogues of curcumin (MACs). *Current pharmaceutical design*. 2013;19(11):2114-35.
- [28] Gruber BM, Tomasz D, Adam K, Irena B. THE INFLUENCE OF POLYETHER SUBSTITUENTS ON BIOLOGICAL ACTIVITY OF CURCUMIN DERIVATIVES. *Acta Poloniae Pharmaceutica-Drug Research*. 2020;77(1):99-111.