Tropical Journal of Pharmaceutical Research June 2026; 25 (6): 797-802
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
https://dx.doi.org/10.4314/tjpr.v25i6.5

Original Research Article

In vitro assessment of the antiproliferative effects of Iraqi *Euryops pectinatus* ethyl acetate extract on MDA-MB-231 breast cancer cells

Fatima Talib Abdullah*, Dhuha A. Al Shammaa
Department of Pharmacognosy, College of Pharmacy, University of Baghdad, Baghdad, Iraq

For correspondence:* ***Email:* *ftalib248@gmail.com*

Sent for review: 29 April 2026 *Revised accepted: 17 June 2026*

Abstract

Purpose: *To investigate the antiproliferative activity of ethyl acetate extract of Iraqi Euryops pectinatus on MDA-MB-231 human breast cancer cells.*
Methods: *The dried plant material was extracted by maceration with solvents of increasing polarity. The ethyl acetate extract was subjected to RP-HPLC for phytochemical profiling. The antiproliferative activity of the extract was assessed by MTT assay on MDA-MB-231 breast cancer cells.*
Results: *Several phenolic and flavonoid compounds such as quercetin, apigenin, rutin, rosmarinic acid, caffeic acid and naringenin were present. Cytotoxic activity of the ethyl acetate extract was concentration-dependent against MDA-MB-231 cells with an IC_{50} value of approximately 2023 µg/mL. Cell viability decreased significantly with increasing extract concentrations, indicating moderate antiproliferative activity ($p < 0.05$). Observed activity may be associated with combined or synergistic effects of the identified phenolic and flavonoid constituents.*
Conclusion: *Euryops pectinatus significantly exhibits cytotoxic activity against MDA-MB-231 breast cancer cells in a concentration-dependent manner, attributed to the synergistic effects of the phytoconstituents.*

Keywords: *Breast cancer, Cytotoxicity, Ethyl acetate extract, Euryops pectinatus, MDA-MB-231*

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Metastatic breast cancer (MBC) is still recognized as a major contributor to cancer deaths among females globally, including the United States [1]. Breast cancer is a highly heterogeneous disease, broadly grouped into four: triple-negative (TNBC), HER2-driven, HER2-enriched, and ER/PR-expressing breast cancer subtypes [2]. Approximately 60-70 % of MBCs are hormone receptor (HR)-positive, which is defined by the presence of progesterone receptor (PR) and/or estrogen receptor (ER) in tumor cells [3]. Surgery, chemotherapy, radiation, endocrine therapy, targeted therapy, and other related modalities are conventional therapies for breast cancer [4].

Throughout history, traditional treatment methods have relied heavily on medicinal plants. Medicinal herbs have long been used in many cultures to treat a range of illnesses, from common ailments such as colds and digestive disorders to more serious conditions including infections, cancer, and those marked by ongoing inflammation [5]. Despite improvements in

traditional therapy, challenges such as drug resistance, toxicity, and recurrence continue to restrict long-term treatment success. Phytotherapy, the use of plant-derived compounds, has emerged as a promising adjuvant therapy for breast cancer [6]. Approximately 10 % of nearly 250,000 plant species in the plant kingdom have been examined or shown to be medicinal for the treatment of numerous ailments, based on current estimates [7,8].

The grey-leaved Euryops, of the Asteraceae family, is an ornamental flowering species indigenous to rocky and sandstone-rich habitats in the Western Cape region of South Africa (from Gifberg to the Cape Peninsula) [9]. The invasive weed *Euryops pectinatus* is a member of the Asteraceae and is frequently referred to as African daisy or Golden Euryops, containing phenols, flavonoids, alkaloids, steroids, saponins, glycosides, and other phytochemicals [10]. Plant-derived bioactive compounds, particularly phenolics and flavonoids, represent key phytochemicals with demonstrated multifaceted biological actions, such as anti-aging, antioxidant, and anticancer effects, as well as their protective roles against cardiovascular and immune-related diseases [11]. Polyphenolic profile of Iraqi Euryops pectinatus has not previously been studied in Iraq. The present study therefore investigated the cytotoxic activity of the ethyl acetate extract of the plant on MDA-MB-231 human breast cancer cell line.

EXPERIMENTAL

Plant collection

Plant specimens were obtained from Babylon Garden for Plant Hobbyists (Babil Hawat Garden, Iraq). The collected material was cleaned, shade-dried at ambient temperature, and mechanically ground into fine powder. A total of 250 g of dried powdered material was macerated using solvents of increasing polarity. Initially, the plant material was extracted with 2.5 L of n-hexane for 3 days, followed by filtration. The plant residue was subsequently extracted with 2.5 L of chloroform for 3 days and then with 2.5 L of ethyl acetate for 3 days. Each extraction step was performed at ambient temperature while stirring. After filtration, the solvents were removed under reduced pressure using a rotary vacuum evaporator. The ethyl acetate extract was selected for further study.

Determination of phytochemical content of the extract

High-performance liquid chromatography (HPLC) was executed on a C18 column and both qualitative and quantitative analyses were conducted. Separation was done with a gradient system of 1 % formic acid in water (solvent A) and acetonitrile (solvent B) with a flow rate of 1 mL/min. Chromatographic peaks were recorded with a diode array detector (DAD). Elucidation of the chemical constituents was carried out by contrasting the retention times of sample peaks with those of respective reference standards under similar chromatographic conditions.

Toxicity testing of ethyl acetate extract of *Euryops pectinatus*

The ethyl acetate extract of *Euryops pectinatus* was applied to MDA-MB-231 cells and cellular viability was quantified using an MTT-based approach. This approach relied on the metabolic competence of viable cells, in which intracellular oxidoreductase enzymes attenuate the yellow tetrazolium compound (MTT) to purple, insoluble formazan crystals.

In vitro cellular cultivation and viability determination

The MDA-MB-231 cells were grown in a controlled environment at 37 °C and 5 % CO_2 in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), penicillin (100 IU/mL), and streptomycin (100 µg/mL) [12]. Before treatment with the ethyl acetate extract, cells were seeded into 96-well plates and allowed to adhere. Formazan crystals were generated by the addition of MTT reagent, followed by continued incubation. A microplate reader was used to detect the absorbance at 570 nm after the crystals were dissolved in dimethyl sulphoxide (DMSO). Cell viability was expressed as a percentage of the untreated control [13]. Concentration-response correlations were used to calculate IC_{50} values [14].

Histological assessment

After 24 – 48 h treatment, morphological features consistent with apoptosis, including cell shrinkage and membrane blebbing, were observed using an inverted microscope at ×40 magnification.

Ethical statement

Ethical approval was not required for this study because the experiments were conducted exclusively on established human breast cancer cell lines (MDA-MB-231) and did not involve human participants, patient-derived samples, or experimental animals.

Statistical analysis

Data were analyzed using GraphPad Prism 8.4.3 (GraphPad Software, USA). Values were presented in mean ± standard deviation (SD) (n = 3). Data integrity checks were done and no outlier was observed. Measurement data were compared using analysis of variance (ANOVA). The IC_{50} values were determined via nonlinear curve fit of dose-response curves and $p < 0.05$ was considered statistically significant.

RESULTS

High-performance liquid chromatography (HPLC) analysis of ethyl acetate extract

The polyphenolic and flavonoid profile of the ethyl acetate extract of *Euryops pectinatus* was resolved by reverse-phase high-performance liquid chromatography (RP-HPLC). The results revealed the presence of gallic acid, rutin, rosmarinic acid, quercetin, apigenin, caffeic acid, naringenin, and luteolin (Figure 1).

Cytotoxic assay

The anti-proliferative activity of ethyl acetate extract of Euryops pectinatus against MDA-MB-231 human breast carcinoma cells was ascertained by colorimetric MTT assay. The findings demonstrated a dose-dependent decline in cell viability, with higher doses of the extract progressively reducing the viable cell population. At 2000 µg/mL and above, a significant decline in cell viability was evident ($p < 0.01$) compared to negative control. Maximum inhibitory (cytotoxic) effect was observed at 8000 µg/mL, at nearly 58 %. The IC_{50} of ethyl acetate extract was approximately 2023 µg/mL, which displayed moderate cytotoxic potential against MDA-MB-231 cells. Conversely, doxorubicin (positive control) displayed a significantly greater cytotoxicity at all doses ($p < 0.0001$) (IC_{50} = 84.66 µg/mL) (Figures 2 and 3). The horizontal axis represented the concentration of the tested extract (µg) and the vertical axis represented cell viability (%). The IC_{50} value was determined from the log–transformed concentration–response curve and was 2023 µg/mL for the ethyl acetate extract.

DISCUSSION

The survival rate of cancer patients is lower due to the development of drug resistance during cancer chemotherapy [15]. Multidrug resistance (MDR) renders cancer cells refractory to standard treatments, posing a major limitation in cancer therapy [16]. To overcome this challenge, many strategies have been proposed, including chemotherapy with a combination of anti-tumor drugs and a P-glycoprotein (P-gp) inhibitor or other ABC transporter inhibitors. P-glycoprotein (P-gp) is the first member of the ATP-binding cassette (ABC) superfamily, which is encoded by the MDR1 gene [16], and is widely expressed in epithelial cells of normal tissues involved in drug disposition (liver, intestine, and kidney).

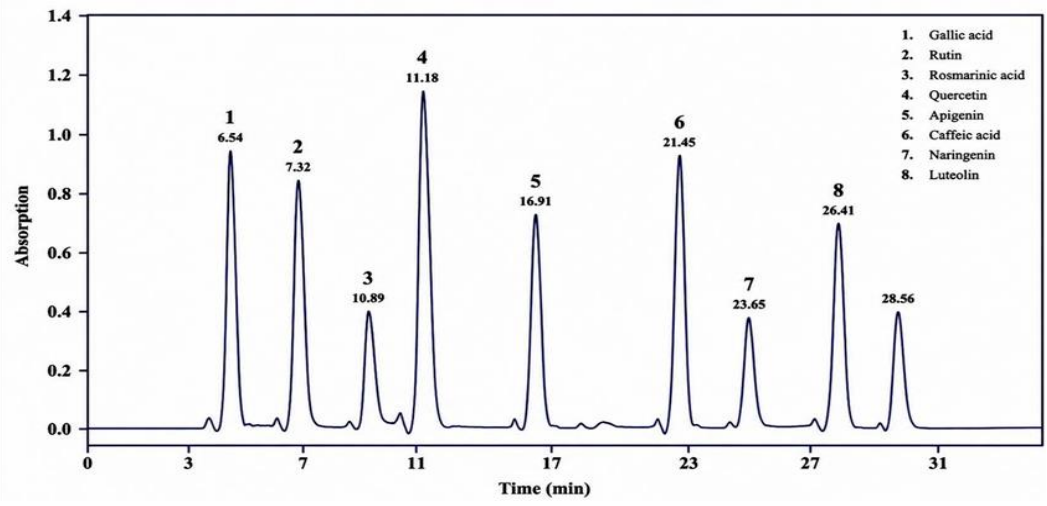

Figure 1: Chromatogram of *Euryops pectinatus* ethyl acetate whole plant extract

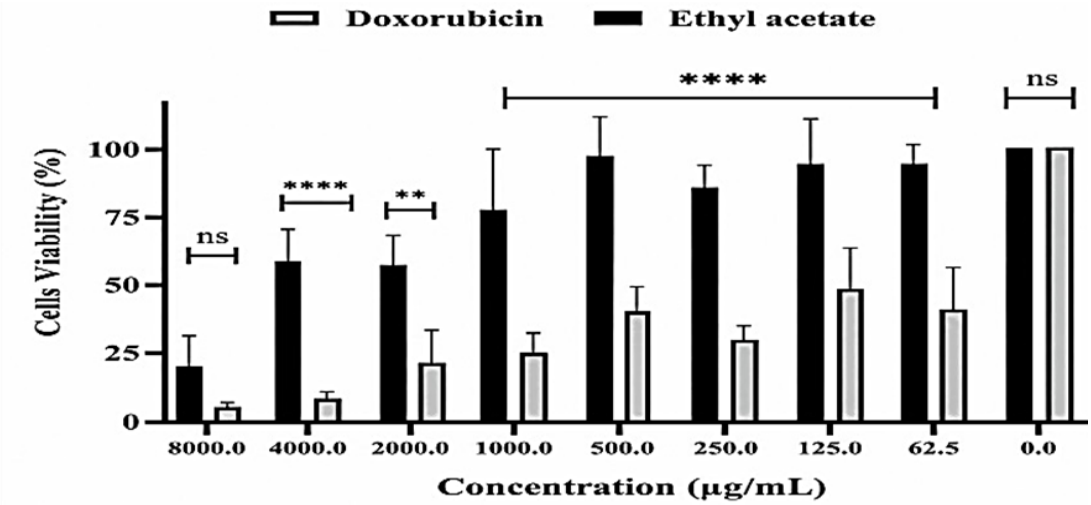

Figure 2: Dose response curve illustrating the influence of the ethyl acetate extract of *Euryops pectinatus* on cell viability. Extract concentrations (µg) are presented on the horizontal axis, while the corresponding percentage of viable cells is plotted on the vertical axis

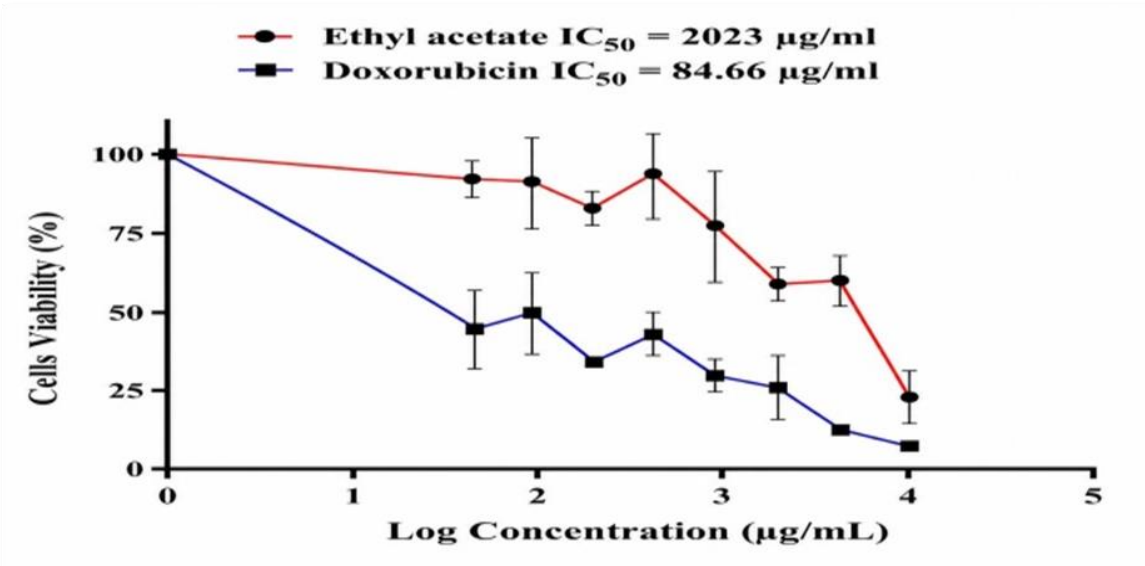

Figure 3: Dose–response profile illustrating IC_{50} value for *Euryops pectinatus* extract

Furthermore, natural compounds isolated from medicinal plants represent promising lead molecules for the development of chemotherapeutic agents [17]. Several studies investigating various parts of Euryops pectinatus have been documented. For example, the hexane extract of E. pectinatus flower-heads was dominated by n-pentacosane in addition to phytosterols in the unsaponifiable fraction, whereas linoleinic acid was the major constituent in the saponifiable fraction [18]. Also, E. pectinatus leaf essential oil showed potent in vitro cytotoxic activity against Ehrlich ascites carcinoma cells (EAC) and human brain cells (U251) [19]. This current study investigated the antiproliferative activity of ethyl acetate extract of Iraqi Euryops pectinatus on MDA-MB-231 human breast cancer cells. The ethyl acetate fraction showed the presence of several phenolics and flavonoids such as gallic acid, rutin, rosmarinic acid, quercetin, apigenin, caffeic acid, naringenin, and luteolin. These findings are consistent with a previous study that revealed high amounts of phenolic and flavonoid compounds in the flower extract [9]. These metabolites have been widely associated with anticancer activity. The observed cytotoxic activity of the plant may be attributable to the high concentrations of flavonoids and phenolic compounds, particularly rosmarinic acid (RA), which was present at higher concentrations than the other identified constituents in the ethyl acetate extract. The concentration of flavonoids was greater than that of phenolic acids in *Euryops pectinatus* ethyl acetate extract.

According to earlier studies, rosmarinic acid (RA) differentially affects cell cycle progression depending on the breast cancer cell line. Rosmarinic acid (RA) was found to induce G0/G1 phase arrest in MDA-MB-231 cells whilst promoting S-phase arrest in MDA-MB-468 cells, which was linked to a significantly increased apoptotic response in MDA-MB-231 cells [20]. Furthermore, it has been demonstrated that RA dramatically raises mRNA expression of pro-apoptotic genes in MDA-MB-231 cells, such as harakiri (HRK), TNF receptor superfamily 25 (TNFRSF25), and BCL2 interacting protein 3 (BNIP3). Synergistic effects of flavonoids in breast cancer models have also been documented. In both MCF-7 and MDA-MB-231 cell lines, blend of quercetin or fisetin with naringenin was observed to boost anti-proliferative and antimigratory effects, indicating that such combinations may offer a promising strategy for future therapeutic development [21]. While the overall potency is moderate compared to doxorubicin, the extract serves as a promising natural pool of bioactive molecules. Future studies could focus on isolating and purifying individual identified components to investigate anticancer synergy.

CONCLUSION

The Iraqi *Euryops pectinatus* ethyl acetate extract showed a concentration-dependent cytotoxic impact on human breast cancer cell line (MDA-MB-231) and may be a viable natural source of anticancer leads. However, further study is needed to identify and isolate the active ingredients and clarify their modes of action.

REFERENCES

1. *Caswell-Jin JL, Sun LP, Munoz D, Lu Y, Li Y, Huang H, et al. Analysis of breast cancer mortality in the US-1975 to 2019. JAMA 2024; 331(3): 233-241*
2. *Shaath H, Elango R, Alajez NM. Molecular classification of breast cancer utilizing long non-coding RNA (lncRNA) transcriptomes identifies novel diagnostic lncRNA panel for triple-negative breast cancer. Cancers 2021;13(21): 5350*
3. *Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024; 74(3): 229-263*
4. *Xiong X, Zheng LW, Ding Y, Chen YF, Cai YW, Wang LP, et al. Breast cancer: pathogenesis and treatments. Signal Transduct Target Ther 2025; 10(1): 49*
5. *Awoke A, Gudesho G, Akmel F, Shanmugasundaram P. Traditionally used medicinal plants for human ailments and their threats in Guraferda District, Benchi-Sheko zone, Southwest Ethiopia. J Ethnobiol Ethnomed 2024; 20(1): 82. doi: 10.1186/s13002-024-00709-5.*
6. *Al Khalily IA, Megantara S, Aulifa DL. Targeting molecular pathways in breast cancer using plant-derived bioactive compounds: A comprehensive review. J Exp Pharmacol 2025; 17: 375-401. doi: 10.2147/JEP.S528132.*
7. *Ijaz S, Akhtar N, Khan MS, Hameed A, Irfan M, Arshad MA, et al. Plant-derived anticancer agents: a green approach towards skin cancers, Biomed Pharmacother 2018; 103: 1643–1651*
8. *Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 2001; 109 Suppl 1(Suppl 1): 69-75. doi: 10.1289/ehp.01109s169*
9. *Elkady MW, Ayoub MI, Abdel-Mottaleb Y, ElShafie MF, Wink M. Euryops pectinatus L. flower extract inhibits P-glycoprotein and reverses multidrug resistance in cancer cells: A mechanistic study. Molecules 2020; 25(3): 647*
10. *Chauhan BS. Grand challenges in weed management. Front Agron 2020; 1: 3*
11. *Nile SH, Park SW. Edible berries: bioactive components and their effect on human health. Nutrition 2014; 30(2): 134-144. doi: 10.1016/j.nut.2013.04.007*
12. *Kao J, Salari K, Bocanegra M, Choi YL, Girard L, Gandhi J, et al. Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery. PLoS One 2009; 4(7): e6146. doi: 10.1371/journal.pone.0006146.*
13. *Salman MI, Al-Shammari AM, Emran MA. 3-Dimensional coculture of breast cancer cell lines with adipose tissue-Derived stem cells reveals the efficiency of oncolytic Newcastle disease virus infection via labeling technology. Front Mol Biosci 2022; 9: 754100*
14. *Freshney RI. Culture of animal cells: A manual of basic technique and specialized applications. 7th ed. Hoboken, NJ: John Wiley and Sons; 2016.*
15. *Persidis A. Cancer multidrug resistance. Nat Biotechnol 1999; 17: 94*
16. *Karthikeyan S, Hoti SL. Development of fourth-generation ABC inhibitors from natural products: A novel approach to overcome cancer multidrug resistance. Anti-Cancer Agents Med Chem 2015; 15: 605–615*
17. *Elkady MW, Ayoub IM. Chemical profiling and antiproliferative effect of essential oils of two Araucaria species cultivated in Egypt. Ind Crops Prod 2018; 118: 188–195*
18. *Nesseem DI, Michel CG. Development and characterization of local anti-inflammatory implantation for the controlled release of the hexane extract of the flower-heads of Euryops pectinatus L. (Cass.) Drug Discov Ther 2011; 5: 96–106*
19. *Michel CG. Essential oil and morphological characters of leaves of Euryops pectinatus (L.) Cass. cultivated in Egypt. Bull Fac Pharm Cairo Univ 2004; 42: 145–158*
20. *Messeha SS, Zarmouh NO, Asiri A, Soliman KFA. Rosmarinic acid-induced apoptosis and cell cycle arrest*

in triple-negative breast cancer cells. Eur J Pharmacol 2020; 885: 173419

21. *Jalalpour CM, Shahbazi S, Reiisi S. Naringenin in combination with quercetin/fisetin shows synergistic anti-proliferative and migration reduction effects in breast cancer cell lines. Mol Biol Rep 2023; 50(9): 7489-7500*