

Review Article

Cytotoxicity and Antioxidant Effect of *Verbena Officinalis* L. Extract on Breast Carcinoma Cell

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ABSTRACT: *Verbena officinalis* L. (*V. officinalis*) is one of the medical plants that plays an important role in the treatment of many diseases. The purpose of this study was to determine the cytotoxic and antioxidant effects of *V. Officinalis* on breast carcinoma cell lines (MCF7). Two pairs of experiments were carried out: The first assessed antioxidant effect of *V. Officinalis* using Scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and the second evaluated the cytotoxicity for the extract on MCF-7 cells were determined by MTT assay. The antioxidant properties of the ethanolic *V. Officinalis*. Extract were effective, with IC₅₀ of 94.12 µg/ml for DPPH radical scavenging. The MTT results revealed anti-cancer activity and viability of cells decrease against MCF-7 cells, with an IC₅₀ of 47.78 µg/ml. In summary, *V. Officinalis*. Ethanolic extract showed strong antioxidant and cytotoxic effects against the MCF-7 cell line.

Keywords: *Verbena officinalis* L., Breast cancer, antioxidant, anticancer.

INTRODUCTION

Breast cancer is a type of malignant neoplasm that is caused when cells in the breast grow out of control and become tumorous. This type of cancer affects more women than any other type of cancer in the world. Moreover, metastasis is the major cause of death for cancer patients [1]. Chemotherapy and radiotherapy are both clinically expensive and can have side effects. In terms of side effects and cost-effectiveness, Medicinal plants may be the most effective alternative to chemotherapy drugs in addressing these concerns. Secondary metabolites with diverse biological activities are abundant in plants [2, 3]. Therefore, they have identified numerous bioactive compounds in plants that have significant therapeutic potential. Antitumor activity is just one of the many medicinal uses discovered for plant chemicals like flavonoids, carotenoids, alkaloids, and phenolics [4,5]. *Verbena Officinalis* L. is one of these plants. It is regarded as one of the medicinal traditional plants found in various countries around the world. *Verbena officinalis*, a plant from the Verbenaceae family, is commonly called pigeons' grass, herb of grace, or vervain. It is a perennial erect herb, which grows to a height of about 25–100 cm, having toothed and lobed leaves. It is a flowering species that belongs to the Verbenaceae family, has medium-sized leaves, and is deciduous [6]. *V. Officinalis* contains glycosides,

flavonoids, sterols, saponins, terpenoids phenolic acids, iridoids, and ursolic, according to the phytochemical qualitative analysis [7]. In vitro studies have shown that this plant's extracts have been employed for the treatment of many ailments such as gastric diseases, abrasion, skin burns, [8] wounds, thyroid problems, rheumatic pain, [9] asthma and cough, [10] amenorrhea, enteritis, acute dysentery, [11] expectorant and diuretic [12]. *V. officinalis* studied for its new important bioactivities including analgesic and anti-inflammatory, [13,14] antioxidant, [15] antifungal, [16] antibacterial, [17,18] anticancer, [19,20] neuroprotective, [11] antidepressant, [21] antiproliferative, [22] and, urolithiasis. [23] properties. *V. Officinalis* extract showed selective anticancer activity. The purpose of this study was to investigate the anticancer and antioxidant activities of an ethanol extract of *V. Officinalis* grown in Iraq in vitro using the human breast cancer MCF-7 cell line.

Experimental Section

Plant Collection and Extraction

Leaf of *V. Officinalis* was collected from Baghdad, Iraq. The collected leaves were thoroughly washed using tap water. Following a thorough washing, the *V. Officinalis* leaves were dried in an incubator at 37 °C for one full night.

A WileyMill grinder was used to grind the leaves into a fine powder (Standard Model 3). Employing the Soxhlet device and the procedure outlined in, [24] An ethanol extract with a purity level of 99.9% was produced. The round bottom flask was filled with 150 mL of ethanol and (50 g) fine powder. After 8 hours of extraction at 70 °C, the solvent extract was concentrated in a rotary evaporator at 40 °C. After collecting the dry extracts, they were weighed and placed in a refrigerator at 4 °C for long-term storage.

DPPH assays

The DPPH free radical scavenging test was used to evaluate the antioxidant activity of the ethanol extract. [25] Spectrophotometry (shimadzu) was used to evaluate the DPPH free radical scavenging activity. Colorimetric changes were measured at 517 nm. For each identified compound, a set of concentrations (25, 50, 100, and 200 µg mL⁻¹) were used in this experiment. As a control, ascorbic acid was used. According to the percentage of free radicals.

$$\%Inhibition = \frac{Abs.of - ve control - Abs.of sample}{Abs.of - ve control}$$

Cell Culture

Michigan Cancer Foundation-7 (MCF-7) was derived from the pleural effusion of a year old female with carcinomas of the breast. [26] Dulbecco's Modified Eagle medium was used to culture the cells, which was supplemented with heat-inactivated foetal bovine serum (10%), streptomycin, penicillin (1%), and glutamine (2 mmol/L).

MTT cytotoxicity assay

V. Officinalis anticancer activity was determined using the MTT assay in the MCF7 cell line [27]. A stock solution of *C. myxa* was prepared by dissolving it in (DMSO), and then serial dilutions (25-200 µg/mL) were made from this solution. After 24 hours in a CO2 incubator, MCF7 cells were treated with extract (100 L) or doxorubicin (control). After 1-4 more hours of incubation, MTT (5 µg/mL) was added to each well. After discarding the media, DMSO was added to each well to dissolve the formazan crystals. Hidex Chameleon microplate reader absorbance was read at 575 nm (LabLogic Systems Ltd., Sheffield, United Kingdom)

Statistical analysis

To determine whether group variance was significant, a one-way analysis of variance (ANOVA) was used. The data were expressed as MeanStandard Deviation (SD), and statistical

significance was determined using GraphPad Prism version 8.02.

RESULT AND DISCUSSION

Antioxidant Activity of *V. Officinalis* Crude Extract

V. Officinalis ethanol extract scavenging activity was determined using DPPH free radical scavenging assays. Results, in **Figure (1)**, shows that the percentage of scavenged free radicals increase, with increasing concentrations of extract. The extract showed significantly high antioxidant activity (70.66% at 200 µg mL⁻¹). (**Figure1**) shows that when the extract's free radical scavenging activity was compared to that of a positive control, ascorbic acid, the two showed nearly identical dose-dependent patterns, at 100 and 200 µg mL⁻¹ concentrations. The basis of this method is the reduction of DPPH solution in the presence of a hydrogen-donating antioxidant. This ability to donate hydrogen atoms or electrons was determined spectro photochemically by bleaching the purple colored DPPH solution. [28] The findings show that *V. Officinalis* is a rich source of antioxidants due to its high flavonoid and phenolic compound content[29].

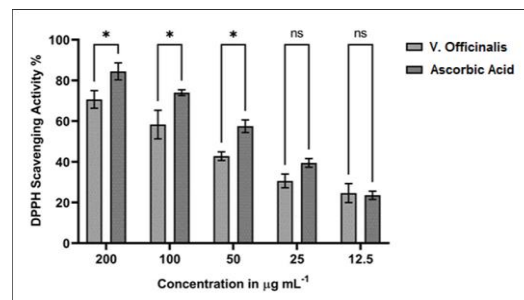


Figure 1: DPPH Scavenging Activity of Ethanolic *V. Officinalis* Extract on Concentrations Compared with Ascorbic Acid.

Viability Assay MTT of *V. Officinalis*.

To evaluate the cytotoxic effects of *V. Officinalis* extract on MCF-7 cells, was used a 3-(dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. For 24 hours at 37 °C., MCF7 cells were exposed to varying concentrations of *V. Officinalis* (200, 100, 50, and 25 µg mL⁻¹). (**Figure 2**) depicts the results of determining the viability of MCF7 cells after treatment with *V. Officinalis*; and it was found to be 42.32%, 54.13%, 65.27%, and 87.43% respectively. *V. Officinalis* cytotoxicity thus increased with increasing concentration, IC₅₀ of 47.78 µg mL⁻¹. Several studies were conducted to determine the chemical content of *V. Officinalis* leaves extract, including qualitative tests for

glycosides, tannins, flavonoids, and phenolics [30]. The extract's therapeutic value as an anti-inflammatory, antiparasitic, antioxidant, and anti-tumor agent was also evaluated [19,21]. The extract of *V. Officinalis* leaves contains a high concentration of flavonoids and other phenolic compounds, which are the main pharmacologically active ingredients [31]. Based on the MTT assay results, *V. Officinalis* is the most potent cytotoxicity and antitumor activity extract against human breast cancer (MCF-7), which is used only in all of the following assay.

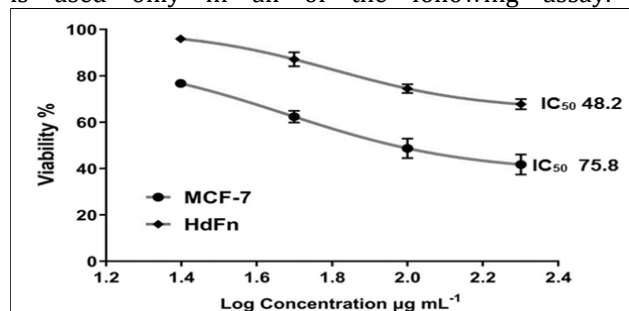


Figure 2: Cytotoxicity of Ethanol *V. Officinalis* extract

CONCLUSIONS

Findings indicated that the ethanolic *V. Officinalis* extract effectively quenched DPPH free radicals. These results suggest that *V. Officinalis* extract may be useful as an anticancer agent because of its high cytotoxicity and antitumor activity against the MCF-7 breast cancer cell line.

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Conflict of interest: None declared.

Ethical approval: The study was approved by the Institutional Ethics Committee of Al-Nahrain University.

REFERENCES

- Abreu, M. T., *et al.* "Bibliography Current World Literature Vol 19 No 4 July 2003." *Current Opinion in Gastroenterology* 19 (2003): 406-441. https://journals.lww.com/co-gastroenterology/fulltext/2003/07000/bibliography_current_world_literature.14.aspx.
- Twaij, Baan Munim, and Md Nazmul Hasan. "Bioactive secondary metabolites from plant sources: types, synthesis, and their therapeutic uses." *International Journal of Plant Biology* 13.1 (2022): 4-14. <https://doi.org/10.3390/ijpb13010003>
- Wawrosch, Christoph, and Sergey B. Zotchev. "Production of bioactive plant secondary metabolites through in vitro technologies—status and outlook." *Applied Microbiology and Biotechnology* 105.18 (2021): 6649-6668. <https://link.springer.com/article/10.1007/s00253-021-11539-w>
- Cosme, Patricia, *et al.* "Plant phenolics: Bioavailability as a key determinant of their potential health-promoting applications." *Antioxidants* 9.12 (2020): 1263. <https://doi.org/10.3390/antiox9121263>
- Habli, Zeina, *et al.* "Emerging cytotoxic alkaloids in the battle against cancer: Overview of molecular mechanisms." *Molecules* 22.2 (2017): 250. <https://doi.org/10.3390/molecules22020250>
- Khan, Abdul Waheed, Arif-ullah Khan, and Touqeer Ahmed. "Anticonvulsant, anxiolytic, and sedative activities of *Verbena officinalis*." *Frontiers in pharmacology* 7 (2016): 231585. <https://doi.org/10.3389/fphar.2016.00499>
- Calvo, M. I., A. San Julian, and M. Fernandez. "Identification of the major compounds in extracts of *Verbena officinalis* L.(Verbenaceae) by HPLC with post-column derivatization." *Chromatographia* 46 (1997): 241-244. <https://link.springer.com/article/10.1007/BF02496313>
- Speroni, E., *et al.* "Effects of differential extraction of *Verbena officinalis* on rat models of inflammation, cicatrization and gastric damage." *Planta medica* 73.03 (2007): 227-235. <https://www.thieme-connect.com/products/ejournals/html/10.1055/s-2007-967116>
- Guarrera, Paolo Maria, Gianluca Forti, and Silvia Marignoli. "Ethnobotanical and ethnomedicinal uses of plants in the district of Acquapendente (Latium, Central Italy)." *Journal of ethnopharmacology* 96.3 (2005): 429-444. <https://www.sciencedirect.com/science/article/pii/S0378874104004453>
- Vitalini, Sara, Franca Tomè, and Gelsomina Fico. "Traditional uses of medicinal plants in Valvestino (Italy)." *Journal of Ethnopharmacology* 121.1 (2009): 106-116. <https://www.sciencedirect.com/science/article/pii/S037887410800562X>
- Lai, Sau-Wan, *et al.* "Novel neuroprotective effects of the aqueous extracts from *Verbena officinalis* Linn." *Neuropharmacology* 50.6 (2006): 641-650. <https://doi.org/10.1016/j.neuropharm.2005.11.009>
- Schönbichler, S. A., *et al.* "Simultaneous quantification of verbenalin and verbascoside in *Verbena officinalis* by ATR-IR and NIR spectroscopy." *Journal of pharmaceutical and biomedical analysis* 84 (2013): 97-102. <https://doi.org/10.1016/j.jpba.2013.04.038>
- Calvo, M. I. "Anti-inflammatory and analgesic activity of the topical preparation of *Verbena officinalis* L." *Journal of ethnopharmacology* 107.3 (2006): 380-382. <https://doi.org/10.1016/j.jep.2006.03.037>
- Calvo, M. I., *et al.* "Anti-inflammatory activity of leaf extract of *Verbena officinalis* L." *Phytomedicine* 5.6 (1998): 465-467. [https://doi.org/10.1016/S0944-7113\(98\)80043-3](https://doi.org/10.1016/S0944-7113(98)80043-3)

15. Bilia, A. R., *et al.* "HPLC–DAD–ESI–MS analysis of the constituents of aqueous preparations of verbena and lemon verbena and evaluation of the antioxidant activity." *Journal of Pharmaceutical and Biomedical Analysis* 46.3 (2008): 463-470. <https://doi.org/10.1016/j.jpba.2007.11.007>
16. Casanova, E., J. M. García-Mina, and M. I. Calvo. "Antioxidant and antifungal activity of Verbena officinalis L. leaves." *Plant foods for human nutrition* 63 (2008): 93-97. <https://link.springer.com/article/10.1007/s11130-008-0073-0>
17. Hernández, Nancy E., M. L. Tereschuk, and L. R. Abdala. "Antimicrobial activity of flavonoids in medicinal plants from Tafi del Valle (Tucuman, Argentina)." *Journal of Ethnopharmacology* 73.1-2 (2000): 317-322. <https://www.sciencedirect.com/science/article/pii/S0378874100002956>
18. Mengiste, Berhan, *et al.* "In vitro antibacterial activity of extracts from aerial parts of Verbena officinalis." *Adv. Biol. Res.* 9 (2015): 53-57. https://www.researchgate.net/profile/Solomon-Abey-2/publication/370934550_In_vitro_Antibacterial_Activity_of_Extracts_from_Aerial_Parts_of_Verbena_officinalis/links/646a458366b4cb4f73c642e1/In-vitro-Antibacterial-Activity-of-Extracts-from-Aerial-Parts-of-Verbena-officinalis.pdf
19. Martino, Laura De, *et al.* "Active caspase-3 detection to evaluate apoptosis induced by Verbena officinalis essential oil and citral in chronic lymphocytic leukaemia cells." *Revista Brasileira de Farmacognosia* 21 (2011): 869-873. <https://doi.org/10.1590/S0102-695X2011005000082>
20. De Martino, Laura, *et al.* "Verbena officinalis essential oil and its component citral as apoptotic-inducing agent in chronic lymphocytic leukemia." *International Journal of Immunopathology and Pharmacology* 22.4 (2009): 1097-1104. <https://doi.org/10.1177/039463200902200426>
21. Lai, Sau-Wan, *et al.* "Novel neuroprotective effects of the aqueous extracts from Verbena officinalis Linn." *Neuropharmacology* 50.6 (2006): 641-650. <https://doi.org/10.1016/j.neuropharm.2005.11.009>
22. Bekara, Amina, Ali Amazouz, and Tahir Benyamina Douma. "Evaluating the antidepressant Effect of Verbena officinalis L.(Vervain) aqueous extract in adult rats." *Basic and Clinical Neuroscience* 11.1 (2020): 91. <https://doi.org/10.32598%2Fbcn.11.1.3>
23. Encalada, Manuel Alejandro, *et al.* "Antiproliferative effect of phenylethanoid glycosides from Verbena officinalis L. on colon cancer cell lines." *LWT-Food Science and Technology* 63.2 (2015): 1016-1022. <https://doi.org/10.1016/j.lwt.2015.03.065>
24. Kumar, Sanjiv, Rajat Sandhir, and Sudarshan Ojha. "Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves." *BMC Research Notes* 7 (2014): 1-9. <https://link.springer.com/article/10.1186/1756-0500-7-560>
25. Patel Rajesh, M., and J. Patel Natvar. "In vitro antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods." *Journal of advanced pharmacy education & research* 1.1 (2011): 52-68. <https://japer.in/storage/models/article/PQbEDKyqpflojyKE3p227GwblZP696ae6jrWe0Rn68zsccttVczBRCVglrLB1/in-vitro-antioxidant-activity-of-coumarin-compounds-by-dpph-super-oxide-and-nitric-oxide-free-radi.pdf>
26. Brooks, Samuel C., Elizabeth R. Locke, and Herbert D. Soule. "Estrogen receptor in a human cell line (MCF-7) from breast carcinoma." *Journal of Biological Chemistry* 248.17 (1973): 6251-6253. [https://doi.org/10.1016/S0021-9258\(19\)43537-0](https://doi.org/10.1016/S0021-9258(19)43537-0)
27. Al-Saffar, A. Z., *et al.* "Phytochemical analysis, antioxidant and cytotoxic potentials of pelargonium graveolens extract in human breast adenocarcinoma (MCF-7) cell line." *Asian Journal of Biochemistry* 12.1 (2017): 16-26. <https://doi.org/10.3923/ajb.2017.16.26>
28. Grases, F., *et al.* "Urolithiasis and phytotherapy." *International Urology and Nephrology* 26.5 (1994): 507-511. <https://doi.org/10.1007/BF02769919>
29. Mošovská, S.; Nováková, D.; Kaliňák M. Antioxidant activity of ginger extract and identification of its active components, *Acta Chimica Slovaca*, **2015**, 8, 115-121. <https://link.springer.com/article/10.1007/BF02767650>
30. Abebe, Mesert, Atakilt Abebe, and Alemayehu Mekonnen. "Assessment of antioxidant and antibacterial activities of crude extracts of verbena officinalis Linn root or Atuch (Amharic)." *Chem Int* 3 (2017): 172-184. <http://bosajournals.com/chemint/article/view/67>
31. Sisay, Mekonnen, Negussie Bussa, and Tigist Gashaw. "Evaluation of the antispasmodic and antisecretory activities of the 80% methanol extracts of verbena officinalis L: evidence from in vivo antidiarrheal study." *Journal of evidence-based integrative medicine* 24 (2019): 2515690X19853264. <https://doi.org/10.1177/2515690X19853264>