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# Cytotoxic Effect of Aqueous-Ethanol Extract of *Typha Domingensis* Pers. (Pollen) against Human Breast Cancer Cells *in Vitro*

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**ABSTRACT**

There is an increasing interest nowadays in using medicinal plants to treat and prevent cancer. This study aimed to investigate the anticancer activity of aqueous-ethanol extract of *Typha domingensis* (Pers) (Pollen), a plant that grows widely in the marsh's areas south of Iraq. The cytotoxic effect was tested on two breast cancer cell lines MCF7 and MDA-MB231 in vitro for 24 hours' exposure. 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was utilized to test the effect of pollen extract on cells proliferation. Results showed that MCF7 cells, estrogen receptor + ($ER^+$), were sensitive ($GI_{50}$ = 254µg/ml) to pollen extract and this effect was confirmed morphologically under microscope, however, MDA-MB231 ($ER^-$) cells were greatly resistant to the same extract. It can be concluded that aqueous ethanolic extract of *Typha domingensis* (Pers) (Pollen) may offer a therapeutic candidate against $ER^+$ breast cancer cells.

**Keywords:** *Typha domingensis* (Pers), pollen, Breast cancer, MCF-7, MDA-MB231, Estrogen receptor.

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## INTRODUCTION

Breast cancer is one of the most popular cancers among women in the world in general and in the eastern Mediterranean region specially in Iraq. The global incidence ratio is 23% among all cancer types that affect females. Moreover, the mortality rate in developing countries is higher than the developed countries due to this disease [1]. Survival rates for breast cancer patients range from 80% or more in North America, Sweden and Japan, to 60% in middle-income countries, and less than 40% in low-income countries [2]. In Iraq, breast cancer accounts for about 32% among other cancer types that affects women according to recent Iraqi cancer registry, which shows that breast cancer ranks first among the cancers that affects the Iraqi population [3]. In recent years, a clear increase in the incidence of this disease has been observed, as local statistics and studies have shown that most cases that affect Iraqi women are usually discovered in late stages that are difficult to control through treatment, and that many of the victims of this disease are in the prime of life [3, 4]. Moreover, radioisotope therapy or chemotherapy are among the prevalent methods of treating various cancerous tumors, however, these methods affect normal cells, causing harmful side effects such as nausea, vomiting, hair loss, as well as suppression of bone marrow function [5, 6]. For this reason, new treatment modalities must be applied to improve clinical outcomes for breast cancer patients. Several crude or purified plant extracts which depicted anti-breast cancer effect both in vitro and in vivo have been reported like *Zingiber officinale* [7], *Morus alba* L. [8] *Angelica archangelica* [9], *Aralia elata* [10], *Pithecellobium dulce* [11] *Abelmoschus esculentus* [12].

*Typha domingensis* (Pers) is one of the most widespread plants in the Mesopotamian marshes south of Iraq with considerable interests in traditional medicine, People of Al-Ahwar (marshes) south of Iraq use pollen powder to raise the male fertility therefore called Viagra of Al-Ahwar [13]. In Turkish folk medicine, the female inflorescences of *Typha* are used externally to cure wounds such as burns. Other than this, pollen is eaten orally in Pakistan as antipyretic, increase flow of urine and to treat injuries [14], leaves have diuretic effect [15] and antimicrobial properties against Gram-negative and Gram positive bacteria [13]. pollen also is a good source of many active ingredients such as Tannins, glycosides alkaloids, saponines, flavonoids and polyphenols [16]. Water extracts of female flowers, male flowers and the fruit of *Typha domingensis* exhibit anti-oxidant and iron chelating activity as well as superoxide and nitric oxide scavenging activities [17].

Although beneficial medicinal applications of this plant have been reported, cytotoxicity against breast cancer cells in vitro has not been described yet. The objective of this study was to evaluate the anticancer activity of aqueous ethanolic extract of Pollen from *Typha domingensis* (Pers) against two human breast cancer cell line models, MCF-7 ($ER^+$) and MDA-MB231 ($ER^-$).

## MATERIALS AND METHODS

Plant Material and Extract Preparation:

The flour of *Typha domingensis* (pers) Pollen was purchased from a local market in Basrah governorate southern of Iraq. Pollen powder was extracted by cold percolation with 75% ethanol (10g /250 ml) for 48 h. The extract was recovered, and 75% ethanol was further added to the plant material and the extraction continued. The two crude extracts were pooled together, and the combined extract was filtered under suction, evaporated using Soxhlet extractor, and kept in aliquots at 4 °C.

Cell culture:

Two human metastatic adenocarcinoma cell lines derived from mammary gland at breast tissue were utilized, MCF7 (Estrogen receptor positive ($ER^+$) [18] and MDA-MB231 (Estrogen receptor negative $ER^-$) [19]. Both cell lines were purchased from VACSERA Co. (Cairo, Egypt). Cells were cultured in RPMI 1640 culture medium (EuroClone, Italy) supplemented with 10% heat inactivated fetal bovine serum (Biowest, South America) in a humidified atmosphere at 37 °C and 5% $CO_2$.

Cytotoxicity assay:

MTT assay was utilized to test the cytotoxic effect of aqueous ethanolic extract on cells proliferation and viability. MTT solution (5mg/ml) was prepared by dissolving MTT powder (Macklin, Shanghai, China) in a

sterile PBS. 50000 cells were seeded for each well prior to exposing cells to the plant extract. Cells were then incubated for 24 hours in 96 well plate to guarantee adherence of cells. Cells were then exposed to increasing doses of plant extract (1µg/ml, 10µg/ml, 100µg/ml, 500µg/ml and 1000µg/ml) for 24 hours in addition to the control vehicle (CV), triplicate wells were used for all treatments. After incubation, media was removed from all wells and washed in PBS. 20µl of MTT solution (5mg/ml) was then added to a similar volume of serum free media into each well. The plate was then incubated in dark for 3 hours at 37 ˚C before adding 50µl dimethyl sulfoxide (DMSO) to dissolve MTT [20]. Plates were then read by microplate reader (Expert Plus reader; Asys Hitech GmbH, Eugendorf, Austria) at 620nm wavelength. Viability percentage was determined using the following formula:

$$Viability\ \% = \frac{A\ test - A\ blank}{A\ control - A\ blank}\ x100$$

where A represents absorbance. Growth inhibitory concentration that reduces viability to 50% ($GI_{50}$) was then calculated from viability curve using GraphPad prism software.

Statistical analysis:

Data collected was analyzed by students` t test and p value less than 0.05 was considered significant. GraphPad prism software was to plot viability curves and calculation of $GI_{50}$ values in addition to students` t-test.

## RESULTS

To assess the cytotoxic effect of aqueous ethanolic extract of *Typha domingensis* (Pers) pollen on two breast cancer cell lines, MCF7 and MDA-MB231 which were incubated with increasing concentrations of extract prior to viability measurement by MTT assay. MCF7 cancer cell line showed sensitivity ($GI_{50}$ = 254µg/ml) after co-culture with aqueous ethanolic extract for 24 hours (Figure 1). The viability percentage in cells treated with high doses (500µg/ml and 1000µg/ml) was reduced significantly greater than 50% (13.9 ± 1.17 and 13.69 ± 3.96 % respectively) in comparison with cells treated with control ($p<0.001$). The pictures in figure 2, were taken directly from culture plates using inverted microscope, also confirmed the inhibitory effect of pollen extract (250 µg/ml) on cellular growth of MCF-7 cells treated for 24 h. This figure (2) depicts the cells irregularity, shrinkage, and detachment in culture treated with pollen extract which indicates anti-proliferative and/or apoptotic activity. However, aqueous ethanolic extract was not effective on MDA-MB231 cells and they were totally resistant even at high doses ($p>0.05$) (Figure 3).

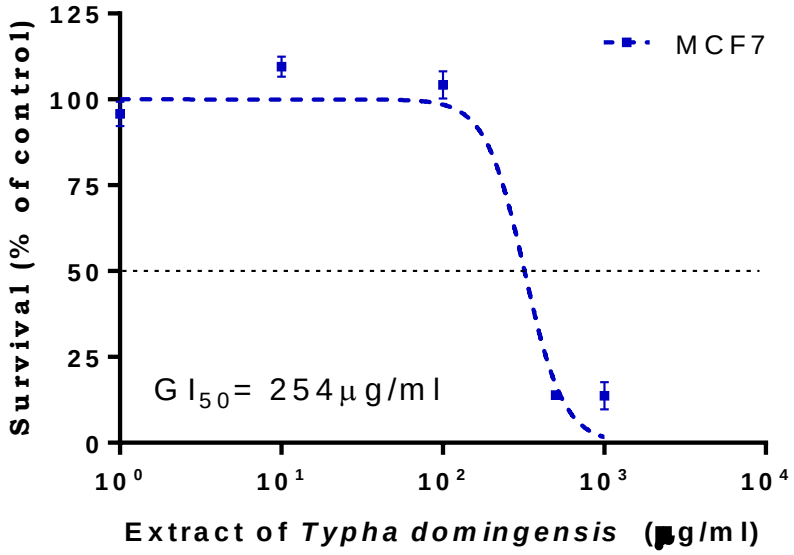


**Figure 1:** Viability of MCF7 breast cancer cell line incubated with increasing concentrations of aqueous ethanolic extract for 24 hours. Nonlinear regression curve fit was plotted by GraphPad prism, $GI_{50}$ was calculated by the same software. Error bars represent the mean viability percentage ± SEM of 2 or 3 replicate wells.


**Figure 2:** Pictures show the effect of incubating MCF7 cells with CV (left) and the $GI_{50}$ concentration of aqueous ethanolic extract (right) for 24 hours, pictures were taken by inverted microscope equipped with digital camera at magnification 100x.

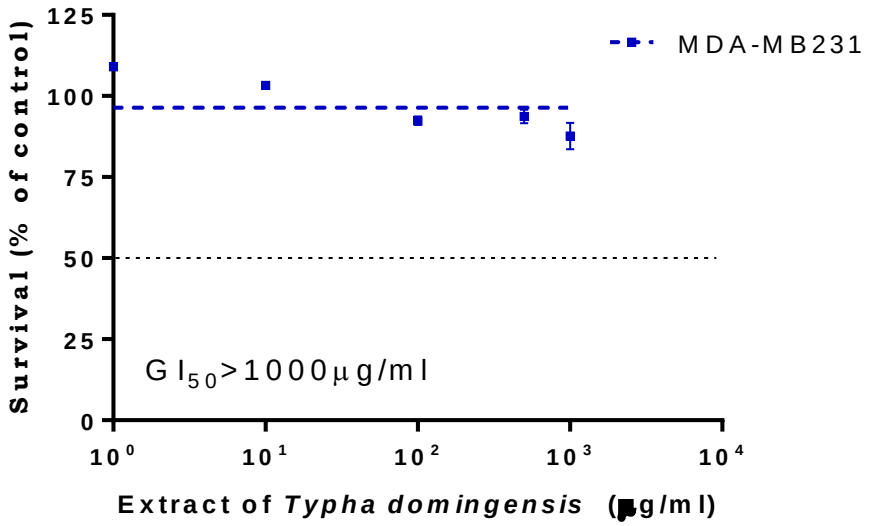


**Figure 3:** Viability of MDA-MB231 breast cancer cell line incubated with increasing concentrations of aqueous ethanolic extract for 24 hours. Nonlinear regression curve fit was plotted by GraphPad prism, $GI_{50}$ were calculated by the same software. Error bars represent the mean viability percentage ± SEM of 2 or 3 replicate wells.

## DISCUSSION

The incidence rates of malignancies, including breast cancer, are expected to rise in spite of the newly developed drugs that combat cancer [21]. Hence, the need to find novel inexpensive, less toxic therapies has become an area of great interest. Herbal extracts have shown various beneficial medical uses and were explored against Haman cancers by using cell lines which are simple and representative models that offer simple and controlled environment to evaluate the anti-proliferative effects [22]. In this study, therefore, the cytotoxic effects of aqueous-ethanol extract of *Typha domingensis* (Pers) were evaluated against two breast carcinoma cell lines which and interestingly, the anti-tumor activity of this extract was not investigated before. It is well established that oxidation stress may play a role in cancer development [23]. Interestingly, it has been reported that multiple plant extracts such as *Calamintha officinalis, Plinia edulis, Cichorium intybus* have shown anti-proliferative effect against breast cancer-derived cell lines with $GI_{50}$ values ranging from 5-500 µg/ml and the main mechanism of action was attributed to the anti-oxidant activity of these plant extracts [24-26]. Here in this study, cytotoxic activity was also observed against MCF7 ($ER^+$) which corroborate the previous findings and this anti-proliferative effect could be attributed to the anti-oxidant and scavenging activity of *Typha domingensis* extract which was previously documented [27]. However, no effect was seen in MDA-MB231 ($ER^-$) cells and this may indicate that the mechanism of cytotoxicity is estrogen receptor-dependent.

**Conclusions**:

In spite that several studies have proven beneficial medical effects for *Typha domingensis* (Pers) however, exploring the anti-tumor potential in vitro of this extract was novel. It can be concluded that the cytotoxic effect seen in $ER^+$ cells (MCF7) may be due to the antioxidant activity and this effect might be associated with ER expression. Hence further studies are recommended to identify the active compounds directly involved in subsequent cytotoxicity both in vitro and in vivo. In addition, exploring the effect of this extract against other cancer types may offer new therapies.

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