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# Antioxidant and Anti-cancer effect of Egyptian and European Pumpkin Seed Oil.

**Sahar Y Al-Okbi[1]*, Doha A Mohamed[1], Eman Kandil[2], Emad K Ahmed[2], and Shaimaa E Mohammed[1].**

[1]Food Sciences and Nutrition Department, National Research Centre, Dokki, Cairo, Egypt.
[2]Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

**ABSTRACT**

The aim of the present research was to study the anti-cancer and *in-vitro* antioxidant activity of Egyptian and European pumpkin seed oil (PSO). The anti-cancer effect of the oils was examined in different human cancer cell lines represented by HepG-2 (liver cancer cells), MCF-7 (breast cancer cells) and Caco-2 (colon cancer cells). Results clarified that Egyptian PSO has significant higher antioxidant activity than the European (105.1 ±8.3 and85.5±7.6, $p<0.05$; respectively). The two oils could inhibit colon cancer cell line with an $IC_{50}$ value of 0.483mg. Egyptian oil showed higher anti-cancer activity than the European towards liver cancer cell while the Egyptian oil had lower activity towards breast cancer cell compared with the European oil.

**Keywords**: Pumpkin seed oils, anticancer, antioxidant.

***Corresponding author***

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## INTRODUCTION

Oxidative stress is among the main causes of many chronic diseases including cancer [1]. Increased levels of reactive oxygen species (ROS) cause oxidative damage are indicated in many forms of cancer like colorectal cancer [2], breast cancer [3], and gastric cancer [4]. High oxidative stress can reduce the body's antioxidant defense against angiogenesis and metastasis in cancer cells. These processes are main factors in the development of cancer [5].Cancer is one of the leading causes of death worldwide [6].Cancer results from uncontrolled cell growth and proliferation caused by mutations in DNA by the carcinogenesis process, or carcinogenic drugs and chemicals, biological (viruses), or physical (radiation) agents. Mutations in DNA convert proto-oncogenes into oncogenes; cell proliferation is increased, and ultimately normal cells are transformed into malignant neoplastic cells. The characteristics of cancer cells include loss of contact inhibition, resistance to apoptosis, and insensitivity to cell growth arrest signals. Angiogenesis is a chief characteristic of cancer cells [7-10].Nutrition and dietary factors represent one of the major factors affects human carcinogenesis. A diet rich in plant food, whole grain, antioxidant and low in saturated fat, such as the traditional Mediterranean diet, is associated with lower risk of cancer [11].

Pumpkin seed oil is among plant foods that contain high level of antioxidant and anticancer ingredients, including β-carotene, unsaturated fatty acids, phenolic compounds, phytosterols and tocopherols. PSO was shown previously to have different health benefits [12-14]

The aim of the present research was to assess the anti-cancer and antioxidant activity of an Egyptian and European PSO variety. The anti-cancer effect of the tested oils was also examined in different human cancer cell line [HepG-2 (liver cancer cells), MCF-7 (breast cancer cells) and Caco-2 (colon cancer cells)].

## MATERIALS AND METHODS

### Materials

*Plant materials*

Egyptian pumpkin seeds (*Cucurbita moschata*, L. Family Curcubitaceae) were purchased from the local market, Cairo, Egypt. European PSO (*Cucurbita pepo*, L. Family Cucurbitaceae var. styria) was obtained from Graz, Austria.

*Cancer cells*

Three human cancer cell lines HepG-2 (liver cancer cells), MCF-7 (breast cancer cells) and Caco-2 (colon cancer cells) were supplied from National Cancer Institute, Cairo University, Egypt.

*Major Chemicals*

Linoleic acid was purchased from Sigma (USA). Petroleum ether 40-60ºC was obtained from BDH Chemical Co, England. All other chemicals and solvents used were of high quality analytical grade.

### Methods

*Preparation of plant materials*

Egyptian pumpkin seeds were dried in an air-circulated oven at 40 °C and reduced into powder.

*Preparation of PSO*

A known weight of the dried Pumpkin seeds powder was placed in a continuous extraction apparatus (Soxhlet) and subjected to extraction using petroleum ether (40-60$^{o}$C) to prepare the oil. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40°C using rotary evaporator.

*Assessment of the bioactivity of PSOs*

*In-vitro Antioxidant Activity of PSOs*

The antioxidant activity of PSO was determined by applying Ferric Thiocyanate (FTC) method according [15, 16] which was slightly modified [17]. PSOs (4mg) or standard (4mg; vitamin E) were separately placed in screw cap tubes and mixed with 4 ml of absolute ethanol, 4.1 ml of 2.52% linoleic acid in absolute ethanol. An appropriate amount of 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water were prepared and added to the previous mixture. The contents of each tube were mixed well and placed in an incubator at 40°C in the dark for a week. To 0.1 ml of this solution, 9.7 ml of 7.5% ethanol and 0.1 ml of 30% ammonium thiocyanate were added. Precisely 3 minutes after an addition of 0.1 ml of 0.02M ferrous chloride that is dissolved in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm. A control was run parallel to the test without the oil samples. The antioxidant activity of each oil sample was carried out in triplicate.

**Calculation:**

The percent inhibition of linoleic acid peroxidation = 100 – [(Absorbance of sample at the seventh day/Absorbance of control at the seventh day) X 100] was calculated to express antioxidant activity.

*Anti-cancer activity of PSOs*

Anti-cancer activity of both PSOs was tested using cell line technique [18]. Cells of HepG-2, MCF-7 and Caco-2 were plated in 96-multiwell plate (104 cells/well) for 24h before treatment with the oils to be attached to the wall of plate. Both oils were dissolved in dimethyl sulfoxide (DMSO) at 10 mM as a stock solution and conserved at 4 °C. Dilutions with culture media were prepared just prior to addition to test plates. Different concentrations of both oils (0, 1, 2.5, 5 & 10 μg/ml) were added to the cell monolayer, triplicate wells were prepared for each individual dose of each oil. Plates were incubated for 48h at 37°C in atmosphere of 5% CO2. After 48h cells were fixed, washed and stained with sulfo-rhodamine-B stain, then excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and different concentration of PSOs is plotted to get the survival curve of each tumor cell line. $IC_{50}$ of each PSO sample (concentration of PSO sample which reduces survival of the exposed cancer cell to 50%) was obtained from the curve.

*Statistical analysis*

Data of antioxidant and anticancer test were expressed as mean ± SE of three determinations. The results of antioxidant effect were analyzed statistically using one-way analysis of variance ANOVA followed by LSD test, $p<0.05$ was used as the criterion of statistical significance.

## RESULTS

**The antioxidant activity of PSOs**

The antioxidant activity of European oil (85.5%) was significantly lower compared to that of the Egyptian variety (105.1%). Both oils were shown to possess significant higher antioxidant activity compared to vitamin E (Table 1).

**The anticancer activity of the Egyptian and European PSOs**

Table, 5 showed the $IC_{50}$values of both Egyptian and European PSOs in different cancer cell types obtained from the curves representing the relation between surviving fraction and different concentration of PSOs (Fig. 1-3, tables 2-4). Both oils were shown to possess antitumor activity against the three types of cancer cell lines. The two types of oil were able to inhibit proliferation of colon cancer cell line (Caco-2) with an equal $IC_{50}$ value of 0.483mg. Egyptian oil revealed higher activity than the European towards liver cancer cell line (HepG2), where its $IC_{50}$ value equals 0.483 mg, while that of the European oil was 0.517mg. On the contrary,

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Egyptian oil showed lower anticancer activity towards breast cancer cell line (MCF-7), with $IC_{50}$of 0.517 mg compared with the European oil that showed $IC_{50}$of 0.483 mg.

**Table 1: Mean antioxidant activity of Egyptian and European oils**

| Tested materials | Antioxidant activity |
|---|---|
| Egyptian PSO | $105.1^{a}$±8.3 |
| European PSO | $85.5^{b}$±7.6 |
| Vitamin E | $63.57^{c}$±0.41 |

Means with different superscript letters are significantly different at P <0.05

**Table 2: Concentration of PSO in relation to HEPG2 surviving cells (Mean±SE)**

| Concentration: mg oil | Surviving fraction of cancer cells treated by European PSO | Surviving fraction of cancer cells treated by Egyptian PSO |
|---|---|---|
| 0.000 | 1.000±0.000 | 1.000±0.000 |
| 0.892 | 0.102±0.009 | 0.125±0.026 |
| 2.229 | 0.090±0.010 | 0.120±0.012 |
| 4.458 | 0.112±0.007 | 0.129±0.008 |
| 8.916 | 0.158±0.024 | 0.196±0.012 |

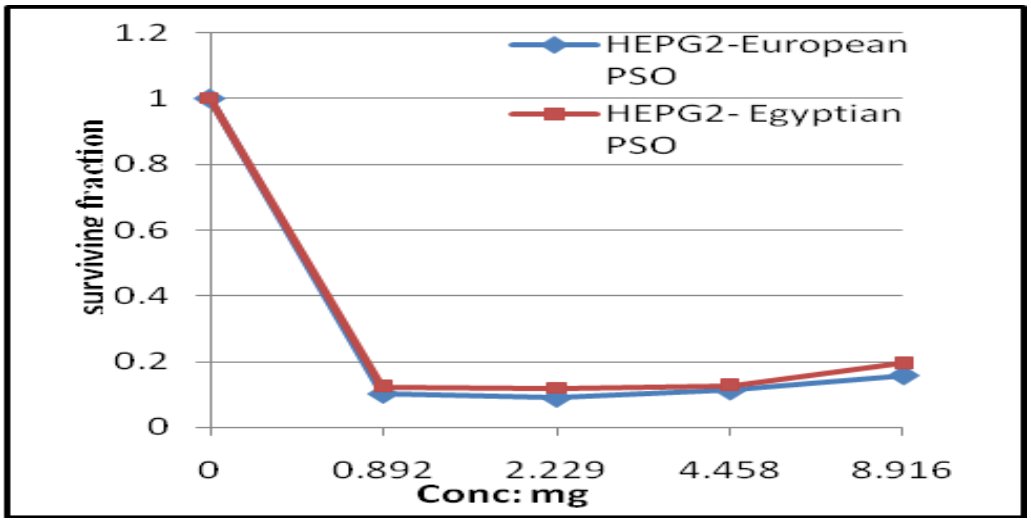


**Figure: Survival curve of liver cancer cell line (HepG2)**

**Table 3: Concentration of PSO in relation to Caco-2 surviving cells (Mean±SE)**

| Concentration : mg oil | Surviving fraction of cancer cells treated by European PSO | Surviving fraction of cancer cells treated by Egyptian PSO |
|---|---|---|
| 0.000 | 1.000±0.000 | 1.000±0.000 |
| 0.867 | 0.115±0.012 | 0.136±0.021 |
| 2.168 | 0.141±0.015 | 0.144±0.029 |
| 4.335 | 0.146±0.011 | 0.156±0.006 |
| 8.670 | 0.191±0.006 | 0.209±0.012 |

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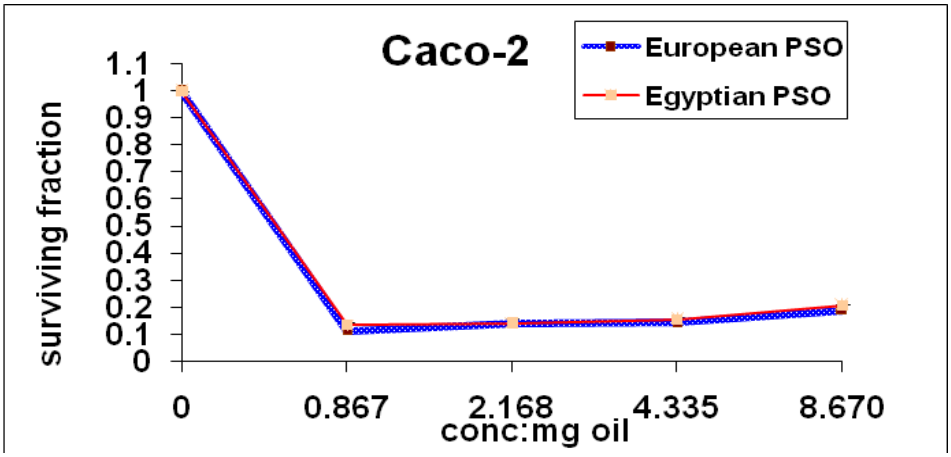


**Figure 2: Survival curve of colon cancer cell line (Caco-2)**

**Table 4: Concentration of PSO in relation to MCF7 surviving cells (Mean±SE)**

| Concentration : mg oil | Surviving fraction of cancer cells treated by European PSO | Surviving fraction of cancer cells treated by Egyptian PSO |
|---|---|---|
| 0.000 | 1.000±0.000 | 1.000±0.000 |
| 0.892 | 0.086±0.001 | 0.094±0.003 |
| 2.229 | 0.091±0.005 | 0.118±0.006 |
| 4.458 | 0.111±0.007 | 0.132±0.008 |
| 8.916 | 0.136±0.009 | 0.197±0.009 |

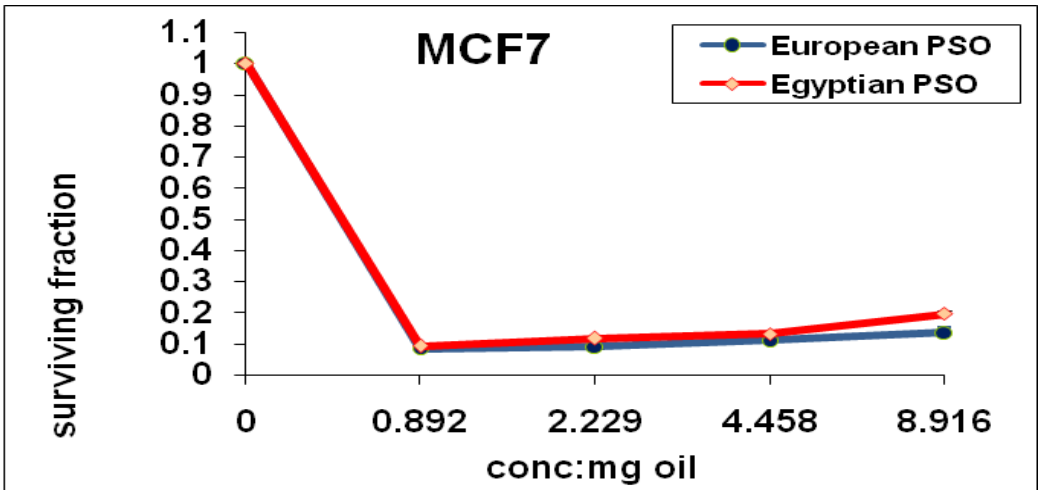


**Fig.3-Survival curve of breast cancer cell line (MCF-7)**

**Table 5: $IC_{50}$ of Egyptian and European PSOs (mg) in different cancer cells**

| PSO | Cancer cell line | | |
|---|---|---|---|
| | Liver cancer cell (**HepG2**) | Colon cancer cell **(Caco-2)** | Breast cancer cell **(MCF-7)** |
| **Egyptian** | **0.483** | **0.483** | **0.517** |
| **European** | **0.517** | **0.483** | **0.483** |

## DISCUSSION

In the present research, PSO from two different sources an Egyptian and European variety was studied to assess different biological activities including *in-vitro* antioxidant, and anti-cancer effect in three different Human cancer cell lines. The antioxidant and anticancer effect of both PSOs might be related to the

contents of functional ingredients such as phenolic content, β-carotene, tocopherols and phytosterols reported to present in PSO [12-14]. In agreement with the present study a previous work [19] showed that PSO possess free radical scavenger effect in rats. Previously, it has been reported that the *in-vitro* antioxidant activity is related to the phenolic content of the plant or extract [20]. Phenolic compounds have been reported to have multiple biological effects including antioxidant, anti-inflammatory and hypocholesterolemic activity [21, 22]. Phenolic antioxidant activity may be related to different mechanisms as hydrogen donation, free radical scavenging and metal ion chelation. Phenolic compounds acting as antioxidants by terminating the free radical chains. They interfere with the oxidation of lipids by the rapid donation of hydrogen atom to radicals (e.g. ROO•+ PhOH→ROOH+ PhO•). Such phenoxy radical intermediates are relatively stable so they can not initiate further radical reactions [23]. The interest in phenolic compounds has increased greatly owing to their antioxidant capacity and their beneficial implications in human health [24]. These include the treatment and prevention of cancer [24]. It was reported that phenolic constituents provide anti-proliferative effect towards cancer growth [25]. It is to be noted that PSO contain phenolic compounds. Epidemiologists have observed that a diet rich in polyphenolic compounds may result in a positive health effect attributed to their antioxidant properties [26].

Carotenoids including β-carotenes can protect membranes, organelles and protein against oxidative damage. It was reported that high β-carotene concentration in fruits and vegetables provides antioxidant properties. β-carotene was found to be one of the most abundant carotenoids in human serum and plants namely pumpkin. Carotenoids present in PSO are known as cellular antioxidants. They are efficient quenchers of singlet oxygen. In addition, β-carotenes react with peroxyl radicals to produce epoxide and apocarotenol products. It was reported previously that β-carotenes inhibit the oxidation of linoleic acid by lipoxygenase with the formation of the hydroperoxide products [27-29].

It was reported that β-carotene produce inhibitory effect on the growth of various human cancer cell lines [30]. It was found that β-carotene can inhibit breast cancer cells proliferation, and increase apoptosis [31].The antioxidant activity of β-carotene arises from the ability of its conjugated double-bonded structure to delocalize unpaired electrons [32]. β -carotene thus show potent ability to quench singlet oxygen, and perform chemical reactivity with free radicals such as the peroxyl ($ROO^{\bullet}$), hydroxyl ($^{\bullet}OH$), and superoxide radicals ($O2^{\bullet-}$) [32, 33]. β -carotene exhibits anti-proliferative effect on various cancer cell lines including colon and leukemic cancer cells. In addition, β-Carotene has been shown to inhibit the expression of anti-apoptotic protein Bcl-2 in cancer cells, reducing thus growth of cancer cells [34]. Such results also could explain the anticancer activity of both oils in the present study towards the three cancer cell types.

Exposure to continual high oxidative stress and inflammation together with genetic factor could lead to cancer. DNA mutations have also some relation to incidence of cancer which could be a result of attack by reactive oxygen species. Chronic inflammation arising from a variety of environmental and infectious sources is associated with promotion of many cancer types [35]**.**It is speculated that antioxidants could protect from the incidence of cancer. However once cancer occur using antioxidants within the cancer cells could lead to enhanced growth of malignant cells.

## CONCLUSION

In the current study PSO of both Egyptian and European sources was found to possess antioxidant and anticancer activity against the three types of human cancer cell lines tested; liver, colon and breast. PSO thus may have a role in controlling tumor proliferation. Anticancer and antioxidant effect of PSO observed in the present study could be attributed to the different functional ingredients present in PSO.

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