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## Evaluation of anti breast cancer activity of some edible plants cultivated in Egypt

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*Origanum majorana* L., *Lupinus albus* L., *Hyphaene thebaica* L. Mart and *Anethum graveolens* L. are edible Egyptian plants used in traditional medicine to treat wide range of ailments. Testing *in vitro* anti-breast cancer activity of these plants. The activity of the ethanolic extract of each plant was tested against MCF-7 breast cancer cell line using Sulphorhodamine-B (SRB) assay. None of these plants possessed significant anticancer activity against MCF-7 cell line. In conclusion, *O. majorana* L., *L. albus* L., *H. thebaica* L. Mart and *A. graveolens* L. have insignificant activity against breast-cancer cell line.

**Keywords:** *Origanum majorana*, *Lupinus albus*, *Hyphaene thebaica*, *Anethum graveolens*, MCF-7, SRB assay

### INTRODUCTION

There is a growing increase in the incidence of breast cancer, and it is the most relevant cancer-related cause of female mortality. In spite of significant progress in the management of breast cancer, the search for a curative treatment is still ongoing (Article, 2017). Natural products, particularly dietary substances containing antioxidant properties, have played an important role in creating new chemopreventive agents, as they possess ability to inhibit carcinogenesis (Surh YJ., 2003).

*Origanum majorana* L. family (Labiatae) popularly known as “Marjoram” is an herbaceous and perennial plant native to southern Europe and the Mediterranean area. They are traditionally used for their biological properties, including antibacterial, antithrombin and antihyperglycemic activities. Marjoram contains phenolic terpenoids (thymol and carvacrol), flavonoids (diosmetin, luteolin, and apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin,

vitexin, orientin, and thymonin) and triterpenoids (ursolic acid and oleanolic acid) (Abdel-massih et al., 2010).

*Lupinus albus* L. family (Fabaceae) popularly known as “White lupin” is non-native, has been used in human nutrition and treatment for several thousand years. Unique traits of protein, fatty acids with a desirable ratio of omega-6 to omega-3 acids, and fibre as well as other specific components, for example oligosaccharides and antioxidants or non-starch carbohydrates, make White lupin an excellent component in many healthy diets. The effects of its components concern the physiological condition of the human body, including diabetes, hypertension, obesity, cardiovascular diseases, lipid concentration, glycaemia, appetite, insulin resistance, and colorectal cancer (Janusz, 2017).

*Hyphaene thebaica* (L.) Mart family (Palmae) popularly known as “Doum” palm tree native to Egypt, sub-Saharan Africa and West India (Hsu et al., 2006). In Egypt, *Hyphaene thebaica* L. Mart

grows commonly in the Nile Valley, especially in Aswan and Qena Provinces (Taylor et al., 2011). In local medicine, *Hyphaene thebaica* L. Mart extracts are being used in the treatment of bilharzia, haematuria, bleeding especially after childbirth and as a haematinic agent (Kamis et al., 2003). It is natural, safe and non-toxic plant could be of great merit for use as a hypocholesterolemic drug (Hetta & Yassin, 2006). Doum is one of commonly consumed beverages in traditional places in Egypt and is rich in polyphenolic compounds (Length, 2009).

*Anethum graveolens* L. family (Apiaceae) commonly known as "Dill" native to South-west Asia or South-east Europe (Shekhawat & Jana, 2010). It has been recognized in different system of traditional medicine for the treatment of different diseases and ailments of human beings. Phytochemical screening of this plant revealed the presence of flavonoids, essential oil, phenolic compounds. It has been reported as antibacterial, antispasmodic, antiulcer, antioxidant, hypolipidemic, genotoxic and with diuretic effect (Review et al., 2011).

This is the first report of testing the anti-breast cancer activity of these plants.

## MATERIALS AND METHODS

### Plant Material

*Origanum majorana* L. herb was cultivated and collected in 2015 from the farm of Faculty of Pharmacy, Cairo University and kindly authenticated by Dr. Abd-Elhaleem A. Mohammed, Horticultural Research Institute, Department of Flora and Phytotaxonomy Researches, Dokki, Giza, Egypt. *Lupinus albus* L. seeds and *Anethum graveolens* L. herbs were collected in 2015 from the farm in Seds city, Beni-Suef Governorate and authenticated by the department of botany, Faculty of Agriculture, Cairo University, Egypt. While, *Hyphaene thebaica* L. Mart fruits were collected in 2016 from the botanical garden in Aswan, Egypt and authenticated by Mrs. Therese Labib, head specialist for plant identification, El-Orman Public Garden, Giza, Egypt. Voucher specimens of each plant are kept in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University.

### Extraction

All plants were shade-dried and coarsely powdered. Standard procedure and analytical grade solvents were used for the extraction. Half

kg of each plant powder was extracted with 70% ethanol. The total ethanol extracts were separately concentrated under reduced pressure and dried to get yield of 75, 90, 125 and 55 g for *O. majorana* L., *L. albus* L., *H. thebaica* L. Mart and *A. graveolens* L., respectively. Each extract was separately stored in sealed amber colored glass container until tested.

### Chemicals

Dimethylsulphoxide (DMSO), RPMI-1640 medium, Trypan blue, Fetal Bovine Serum (FBS), Penicillin/Streptomycin antibiotic and Trypsin-EDTA (Sigma-Aldrich), Tris buffer (AppliChem) were used. All chemicals and reagents used in this study are of highest analytical grade.

### Cell Line

Human breast tumor carcinoma cell line (MCF-7) used in this study was obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The tumor cell line was maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Samples were prepared by dissolving 1:1 solution and stored at -20°C in DMSO at 100 mM.

### Anticancer activity (SRB assay)

The assay was carried out according to the method of (Skehan et al., 1990). The cells were plated in 96-multiwell plate (10<sup>4</sup>cells/well) for 24 hrs before treatment with the extracts to allow attachment of cell to the wall of the plate. Different concentrations of the extract under test (0, 1, 2.5, 5 and 10µg/ml in DMSO) were added to the cell monolayer triplicate wells and prepared for each individual dose. Monolayer cells were incubated with the extract for 48 hrs at 37°C and in an atmosphere of 5% CO<sub>2</sub>, and then the cells were fixed, washed and stained with SRB stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader at 564 nm and the mean background absorbance was automatically subtracted. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified extract. IC<sub>50</sub> (dose of extract which reduces survival to 50%) was calculated for each extract and cell line.

## RESULTS

The anticancer effect of ethanol extracts of *O. majorana* L., *L. albus* L., *H. thebaica* L. Mart and *A. graveolens* L. on MCF-7 cell line were tested

by SRB assay. Different concentrations of the extract were used 5, 12.5, 25, 50  $\mu\text{g/ml}$  and  $\text{IC}_{50}$  was calculated for each extract. Results of anticancer activity against MCF-7 cell line of the tested samples are presented in Figure 1. All extracts showed insignificant activity against breast-cancer MCF-7 cell line with  $\text{IC}_{50}$  greater than 100  $\mu\text{g/ml}$ .

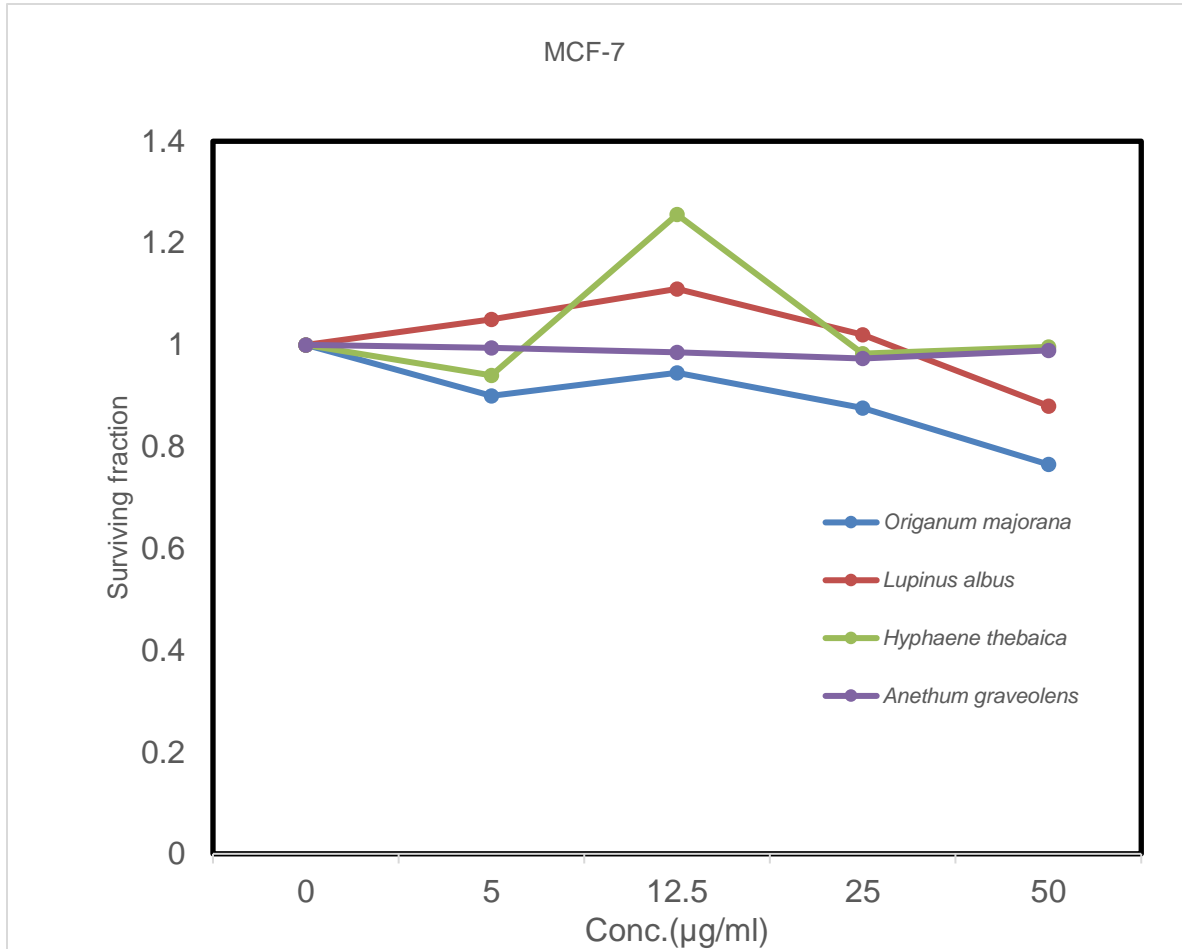


Figure 1: Anticancer activity of *O. majorana*, *L. albus*, *H. thebaica* and *A. graveolens* against MCF-7 (breast cancer) cell line.

## DISCUSSION

Some edible Egyptian plants are reported for their antioxidant activity; which could be correlated for their investigation as anticancer agents. In this study we tested *in vitro* anticancer activity of ethanol extracts of *O. majorana* herb, *L. albus* seed, *H. thebaica* fruit and *A. graveolens* herb cultivated in Egypt by SRB assay.

Literature survey supports the presence of phenolic compounds in these plants, which are compounds reported to be responsible for the antioxidant and antiproliferative activities. Natural antioxidants inhibit tumor growth selectively due to the difference in redox status of normal cell and cancer cell (Sudjaroen, 2014). In the present study, all extracts were found insignificant against MCF-7 (breast cancer) cell line with IC<sub>50</sub> greater than 100 µg/ml. According to U.S. National Cancer Institute (NCI) guidelines, the limit of activity for crude extracts is IC<sub>50</sub> less than 30 µg/mL after 72 hrs of exposure. However, a crude extract with IC<sub>50</sub> less than 20 µg/mL is considered highly cytotoxic (Vijayarathna and Sasidharan, 2012). The IC<sub>50</sub> of the plants under investigation were found to be above the limits specified by NCI, USA for categorization of compound as chemotherapeutic. Therefore, *O. majorana* herb, *L. albus* seed, *H. thebaica* fruit and *A. graveolens* herb have insignificant potential against MCF-7 cell lines. This study will be helpful to avoid any repeated study in this direction in the future.

## CONCLUSION

The ethanol extracts of *O. majorana* herb, *L. albus* seed, *H. thebaica* fruit and *A. graveolens* herb have insignificant potential against MCF-7 cell line, after evaluation by SRB assay.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

MMM collect and extract plants, analysis the data of the experiment and wrote the manuscript. MHH reviewed all data and experiment and reviewed the manuscript. AIO, MAR reviewed the manuscript.

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