AIN SHAMS UNIVERSITY
Faculty of Science
Zoology Department

Evaluating and Comparing the Anti-tumour Effects of Alcoholic Extract and Some Bioactive Components of Sweet Marjoram (*Origanum majorana* L.) on Colon Cancer Cell Line

A Thesis

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ABSTRACT

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Colorectal cancer (CRC) is one of the most frequent malignant tumours worldwide. Nowadays, complementary and alternative treatment strategies have become necessary to improve the survival rate of the CRC patients. Therefore, the present study investigated the anticancer effects of different concentrations of the Egyptian sweet marjoram (*Origanum majorana* L.) methanolic extract (ME) and some of its bioactive components (carvacrol, CA and ursolic acid, UA) on the HCT116 colon cancer cell line. The data of the present study indicated that ME, CA and UA were specifically able to inhibit proliferation and induce cytotoxicity in the HCT116 cells compared with the non-cancerous human skin cell line (BJ-1). Based on the IC_{50} values and the selectivity index (SI), three concentrations (0.25, 0.5 and 1.0 of the IC_{50} at 48 hours of cell culture incubation) were used in the present study to evaluate the possible anticancer molecular mechanisms of the used natural products. Our results proved that ME, CA and UA induced morphological alterations related to apoptosis, inhibited cell adhesion and migration, and reduced spheroids' volumes of the HCT116 cells after 48 hours of treatment in a concentration-dependent manner.

Also, they triggered the DNA fragmentation and increased the percentage of apoptotic cells in sub-G1 phase in the HCT116 cells, which accompanied by decreasing the total antioxidant capacity along with increasing the tumour suppressor p53 protein and the release of mitochondrial cytochrome C into the cytosol. In addition, they upregulated the expression of death receptors (Fas/CD95 and tumour necrosis factor recptor-1) and increased caspases 3, 8 and 9 in the HCT116 cells. All of these data indicated the ability of these natural products to significantly trigger both the intrinsic and extrinsic pathways of apoptosis in cancer cells. In general, the highest anticancer activity in the present study was achieved by UA followed by CA, rendering these compounds may become promising anticancer agents in treating CRC patients in the near future.

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LIST OF ABBREVIATIONS

A_B	Absorbance of blank
ANOVA	Analysis of variance
Apaf1	Apoptotic protease activating factor-1
A_S	Absorbance of sample
ATCC	American Type Culture Collection
Av_{NC}	Averages of the absorbance of the negative control
Av_S	Averages of the absorbance of the sample
Bax	Bcl-2 associated X protein
Bcl-2	B-cell lymphoma-2
Bcl-xL	Bcl extra-large
Bid	BH3 interacting-domain death
bp	Base pair
BSA	Bovine serum albumin
CA	Carvacrol
CCD	Charge-coupled device
CCM	Complete culture media
Cdks	Cyclin-dependent kinases
cDNA	Complementary DNA
Cox	Cyclooxygenase
CRC	Colorectal cancer
Ct	Threshold cycle
d	Diameters
DMEM/F12	Dulbecco's Modified Eagle Medium: Nutrient Mixture F12
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DPA	Diphenylamine assay

EDTA	Ethylene-diamine-tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
Fas	First apoptosis signal (CD95 death receptor)
FasL	Fas Ligand
FBS	Foetal bovine serum
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
GRAS	Gained generally regarded as a safe
H_2O_2	Hydrogen peroxide
hr	hours
HRP	Horseradish peroxidase
JNK	Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
ME	Methanolic extract of Egyptian sweet marjoram
MMP	Matrix metalloprotease
M-MuLV	Moloney murine leukaemia virus
MTT	Tetrazolium dye
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B-cells
PAGE	Polyacrylamide gel electrophoresis
Akt	Protein kinase B
PARP	Poly (ADP ribose) polymerase
PBS	Phosphate buffered saline
PI3K	Phosphatidylinositol-3-kinase
poly-HEMA	Poly-2-hydroxyethyl methacrylate
PVDF	Polyvinylidene difluoride
qPCR	Quantitative (real-time) polymerase chain reaction
RIPA	Radioimmunoprecipitation assay
RNA	Ribonucleic acid
ROS	Reactive oxygen species