

**BIOTECHNOLOGICAL DETECTION OF SOME
MARKER GENES IN CANCER CELL AND THEIR
RELATION TO NATURAL ANTICANCER
COMPOUNDS**

By

MUSTAFA TAREQ MOAWAD

B.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2011

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ABSTRACT

Leukemia is a rapidly progressive disease which is one of the most common causes of death worldwide. Several therapy modalities have been employed in the treatment of leukemia such as natural anticancer compounds which found in plants e.g. *Saussurea Lappa* (*S. lappa*).

Cytotoxic activity of *S. lappa* extracts was determined in relation to CCAAT/Enhancer Binding Protein Alpha (C/EBPA) gene expression, in addition to phytochemical analysis and antioxidant activity.

Successive extraction of *S. lappa* root powder using methanol, ethanol, acetone, ethyl acetate and chloroform solvents was done; the twelve fractions were separated from chloroform extract by thin layer chromatography (TLC). Estimation of total phenolic and total flavonoid was done together with antioxidant. Cytotoxic activity of *S. lappa* extracts and twelve fractions which have been tested on three leukemia cell cultures (CML, AML, ALL) by MTT assay. C/EBPA gene expression was detected by quantitative real time PCR (qRT-PCR).

The chloroform extract of *S. lappa* showed the highest cytotoxic activity against three cancer cell cultures. Regarding C/EBPA gene expression in leukemic cells after addition of chloroform fractions (C2+C9) from the *S. lappa* plant, a statistically significant difference (P value 0.008) with (C9) compound was found this denotes the higher cytotoxic activity of (C9) on the leukemia cells.

The *S. lappa* roots contain phenol and flavonoid compounds, which have a high cytotoxic effect on leukemic cells and improve C/EBPA gene expression which is a known good prognostic marker. Further studies of *S. lappa* for the elucidation of the cytotoxic mechanisms considered as immense importance to deal with leukemia must be done in the future.

Keywords: *Saussurea lappa*, phytochemical analysis, antioxidant, cytotoxic activity, C/EBPA gene

Dedication

I dedicate my dissertation work to my family and my wife and my friends. A special feeling of gratitude to my loving parents, Tareq and Reda whose words of encouragement and push for tenacity ring in my ears. My brothers Amr, Hussien and Abd el rahman have never left my side and very special.

I dedicate this work and give special thanks to my loving wife Kariman and my children Seif and Tareq whose support, enthusiasm and tolerance enabled me to complete the work.

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LIST OF ABBREVIATION

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxy toluene
C/EBPA	CCAAT/enhancer-binding protein alpha
CE	Costunolide
CML	Chronic myeloid leukemia
DE	Dehydrocostuslactone
DPPH	(2, 2-diphenyl-1-picrylhydrazyl)
DW	Dry weight
ECM	Extracellular matrix
GAE	Gallic acid equivalent
iNOS	the inducible nitric oxide synthase
MMPs	Matrix metalloproteinase
MTT	(3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
PBS	phosphate buffer saline
PC	Positive control
Ph	Philadelphia
Ph.th	Phenolphthalein
QE	Quercetin equivalent
ROS	Reactive oxygen species
RT-PCR	Real time polymerase chain reaction
TFC	Total flavonoid contents
TLC	Thin layer chromatography
TNF- α	Tumor Necrosis Factor alpha
TPA	12-O-tetradecanoylphorbol-13-acetate
TPC	Total phenolic contents
Wt.	Weight
ppm	Part of million

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