USE OF MOLECULAR BIOLOGY FOR STUDYING THE MODE OF ACTION OF SOME MEDICINAL PLANT EXTRACTS AS ANTICANCER AGENTS

By

ABEER SAYED KANDIL IBRAHIM KANDIL
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2012

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Agricultural Biochemistry)

Department of Agricultural Biochemistry
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APPROVAL SHEET

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ABSTRACT

Medicinal plants have been used in folk medicine for thousands of years as natural products which play a role in pharmaceutical biology. Therefore, highlighting of the antioxidant and anticancer activities of *Aloe vera, Sonchus oleraceus, Morus alba* and *Cichorium intybus* were the aim of this study. Total phenol and flavonoid content were calculated. Antioxidant activity was determined by DPPH and reducing power using different concentrations. The highest effect for antioxidant activity were estimated for *Morus alba*. The anticancer activity for cancer cell lines was evaluated by MTT assay in MCF-7 breast cancer cell and AML leukemia cancer cells. The ethyl acetate extract of *M. alba* possessed the anticancer activity 78.77% at 250 µg/ml against AML cells. The methanolic extract of *C. intybus* had high effect against MCF-7cell line which was 88.38% at 750 µg/ml, while methanolic extract of *M. alba* recorded 68.68% at 250 µg/ml. Three bioactive fractions were isolated from ethanolic extract of *C. intybus* using TLC (F1-F3). Pure fraction F1 had the highest anticancer activity against breast cancer cells (94.18 ±3.93 %) at 40 µg/ml and F3 gave 62.37±0.66% at 60µg/ml. The expression of related apoptosis genes Bax–BCL-XL was determined. The data showed that the expression of BAX was increased from 1to 2.62 and 1to 1.63 in breast cancer cells after treatment with *C. intybus* and *M. alba* extracts respectively while the expression of BCL-XL was decreased after treatment by both extracts.The data showed that the expression of CEBPA increased in AML cells after treatment by methanolic extracts of *C. intybus* and *M. alba* but with no significant difference between the two extracts.

Key words: *Aloe vera, Sonchus oleraceus, Morus alba , Cichorium intybus,* Antioxidant, Anticancer; MCF-7 cell line, AML cells, CEBPA gene,apoptosis, TLC technique

DEDICATION

I dedicate this work to my lovely mother, for her love and all the support along my life.

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ABBREVIATIONS

AML	Acute Myeloid Leukemia
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
DW	Dry weight
FBS	Fetal bovine serum
FW	Fresh weight
GAE	Gallic a cid equivalent
MCF-7	Michigan Cancer Foundation-7
mg/g DW	Milligram/gram Dry Weight
mg/g FW	Milligram/gram Fresh Weight
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide)
QE	Quercetin equivalent
ROS	Reactive Oxygen Species
TF	Total Flavonids
TP	Total Phenols

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Cancer is a danger as disease that can influence any part of the body, There is over ten million new cases of cancer (all sites excluding non-melanoma skin), with over six million deaths, worldwide. The breast cancer is commonly occurred among women between age group of 45–55, worldwide countries and is mainly attributed to the role of lifestyle and increasing in cancer risk in associated with elevated levels of endogenous and exogenous estrogens in the body (Clemons and Goss, 2011).

Many of studies has been recognized drugs which derived from natural compounds such as alkaloids, phenylpropanoids, and terpenoids (Park *et al.*, 2008) and the US Natural Cancer Institute (NCI) supported these natural compounds as potent anti-cancer drugs in 1950. As examples Vinblastine, vincristine, taxol and camptothecin have improved the chemotherapy of some cancers (Cragg and Newman, 2005).

Morus alba Linn belongs to family *Moraceae*, its leaves have a great importance in folk medicine such as Korea and Japan for diabetes mellitus patients (Katsube, 2006) , as well as roots are used for various health benefits and ailments in Chinese traditional medicines (Chang *et al.*, 2011). It possess phytochemical constituents and biological activities, such as antioxidant, antiviral, anti-inflammatory, hypolipidemic, anti-hyperglycemic, neuroprotective (Pan and Lou

2008), anti-HIV, anti-hypotensive and cytotoxic activities of different species of *Morus* (Du *et al*., 2003).

Cichorium intybus belongs to the *Asteraceae* family, it is a traditional plant which distributed and used as food and medicinal crop throughout Asia and Europe. It has many applications in food and pharmaceutical industries as a carminative and against cardiac ailments (Vilkhu *et al*., 2008).

Chicory possesses anti-cancer (Hazra 2002) anti-fungal and anti-malarial (Bischoff, 2004) anti-diabetic (Pushparaj *et al.*, 2007) and free radical scavenging activity (Lante *et al*., 2011). It is effective in jaundice, asthma, gout and rheumatic complaints (Malik *et al*., 1989) and has the ability to neutralize various free radicals, as well as a linear correlation between phytochemical content and antioxidant capacity of this vegetable (Llorach *et al*., 2004; Lante *et al.*, 2011).

Aloe barbadensis miller (*Aloe vera*) is a member of the *Liliaceae* family and its extract has many biological activities such as hypoglycemic, hypolipidemic, antifungal, anticancer, antioxidant and immunoprotective properties (Choi and Chung, 2003; Yu *et al.*, 2009). *A.vera* gel possesses chemo-preventative and anti-genotoxic affects on benzo[α]pyrene- DNA adducts (Kim and Lee 1997; Lewis *et al*., 2012). *Aloe* gels have also anti-ulcer activitiy which due to several possible mechanisms including its anti-inflammatory properties, healing effects, mucus stimulatory effects and regulation of gastric secretions (Suvitayavat *et al*., 2004).

S. oleraceus L. (family of *Asteraceae*), is a herb native to Europe, North Africa, and Asia and commonly traditional medicinal plant which used as a treatment for warts, ulcers, spider bites and other inflammatory conditions , enteritis, diarrhea, pneumonia, hepatitis, appendicitis, chronic bronchopneumonia, icterus, throat swelling, haematemesis and uraemia (Medica, 1977).

Mehanolic leaf extract of *S. oleraceus* possesses antioxidant compounds (Guil-Guerrero *et al.,* 1998; McDowell 2011) such as: caftaric acid, chlorogenic acid and chicoric acid, chicoric acid have the highest concentration and have high antioxidants activity (Ou *et al.*, 2013).

The aim of this study is to determine the antioxidants and anticancer activities of selected extracts for four plants (*C. intybus*, *S. oleraceus*, *A. vera* and *M. alba* Linn) against MCF-7 cell line and AML cells. As well as, the evaluation was the effect of methanolic extracts of *C. intybus* and *M. alba* at molecular level in those by qPCR technique. The effect of methanolic extract of *C. intybus* and *M. alba* on Bax and BCL-xL gene expression was studied in a trying to know the possible anticancer mechanism of these extracts. In addition, the separation and fractionation of the active ingredients of ethanolic extract of *C. intybus* by TLC technique and tested the anticancer activity of ingredients against MCF-7 cell line at three concentrations.

In addition, the expression of CEBPA gene was determined on proliferation of AML after treatments with *C. intybus* and *M. alba* methanolic extracts.