

Large scale screening of selected Egyptian plant extract's against human colon, liver, breast and lung carcinoma cell lines for detection of antitumor activity *in-vitro*.

Thesis

Submitted to the Faculty of Science Ain Shams University, Cairo, Egypt For the Award of the Ph. Degree in Zoology (Physiology)

By

Ahmed Abd El-Fattah Ibrahim Soliman Pharmacognosy Department, National Research Center, Doqqi- Giza

Supervisors Prof. Dr. Nefissa H. Meky

Professor of Physiology

Faculty of Science, Ain Shams University

Late Prof. Dr. Gamila Mahmoud Wassel

Prof. of Pharmacognosy

Pharmacognosy Department, National Research Center

Late Prof. Dr. Bassem M.S. El-Menshawi

Prof. of Pharmacognosy

Pharmacognosy Department, National Research Center

Dr. Salwa Mohamed EL-Hallouty

Assistant professor

Pharmacognosy Department- National Research Center



Large scale screening of selected Egyptian plant extract's against hulan colon, liver, breast and lung carcinoma cell lines for detection of antitumor activity *in-vitro*.

Thesis Advisors

Prof. Dr. Nefissa Hussein Abd El-Rheem Meky Professor of Physiology, Zoology Deparment, Faculty of Science, Ain Shams University

Late Prof. Dr. Gamila Mahmoud Wassel Professor of Pharmacognosy, Pharmacognosy Deparment, National Research Center

Late Prof. Dr. Bassem M.S. El-Menshawi

Professor of Pharmacognosy, Pharmacognosy Deparment, National Research Center

Dr. Sallwa Molamed EL-Hallouty

Assistant professor Pharmacognosy Deparment National Research Center

APPROVAL SHEET

Large scale screening of selected Egyptian plant extract's against human colon, liver, breast and lung carcinoma cell lines for detection of antitumor activity *in-vitro*.

Ph.D. Degree IN Zoology (Physiology)

By

Ahmed Abd El-Fattah Ibrahim Soliman M.S.c (Zoology), Fac. Sci., Ain Shams Univ., Egypt, 2011.

APPROVAL COMMITTEE

Prof. Dr. Wafaa Ghoneim Shoush Professor of Biochemistry, Fac. Sci., Helwan University

Prof. Dr. Nefissa H. Meky Professor of Physiology, Fac. Sci., Ain Shams University

Prof. Dr. Mohamed Islam Ahmed Fahmy Head of vital application department - Atomic Energy Authority

Date: / /

Akcnowledgement

Before all, limitless gratitude to ALLAH Who blessed my effort and showed me the way.

I wish to express my deep gratitude and appreciation to prof. Dr. Nefissa H. Mekey, prof. Of Physiology, Zoology Department, Faculty of Science, Ain Shams University for sponsoring the thesis and for valuable advices.

My sincere appreciation is also due to Late Professor Dr. Gamila Mahmoud Wassel, Professor of Pharmacognosy, National Research Center, for supervising this study, suggesting the research point and approach. I would like also to express my gratitude for his daily guidance.

My sincere appreciation is also due to Late Professor Dr. Bassem M. S. El-Menshawi, Professor of Pharmacognosy, National Research Center, for supervising this study, suggesting the research point and approach. I would like also to express my gratitude for his daily guidance.

I am sincerely grateful to Dr. Sallwa Mohamed EL-Hallouty. Pharmacognosy Deparment, National Research Center. For suggesting the subject, great scientific help, valuable guidance and indispensable helps given through all the stages of this work

I would like to express my gratitude to Dr. Walid Mohamed fayad. Pharmacognosy Department, National Research Center, for planning the subject, his great scientific help, valuable guidance during the practical work and handwriting of this manuscript.

I would like to express my deep thanks and gratitude to Dr. Khaled Mahmoud Hanafy. Pharmacognosy Department, National Research Center, for his sincere guidance.

]A special Thank you is due to all my colleagues at the in vitro Bioassay- Cell Culture Lab and Pharmacognosy Research Laboratory-NRC for their help and sincere support throughout the years.

Many thanks to the staff members and Head of Zoology Department, Faculty of science, Ain Shams University for their help in this work.

This study was carried out at the Department of Pharmacognosy, National Research Centre, Dokki – Cairo, under project in title:

"Evaluation of some Egyptian plant extracts with preliminary antitumour activity (Comprehensive Biological and phytochemical Studies)", funded by Science & Technology Development Fund (STDF), Academy of Scientific Research and Technology, Egypt, (2013-Current) grant number 1206.

Special deep appreciation is given to my father, my mother, my wife, my brothers and sisters. Also I feel deeply grateful to my dear country Egypt.

TABLE OF CONTENTS

Aknowledgement I	
Abstract II	
List of tables IV	
List of figure VII	
CHAPTER 1	
1- Introduction	1
CHAPTER 2	
2- Review of Literature	
2.1. Pathophysiology of cancer6	
2.1.1. Treatment of cancer72.1.2. Classification of chemotherapeutic agents82.1.2.1.Alkylating agents8	
2. 1.2.2. Antimetabolites	
2.1.2.3. Antitumor antibiotics	
2.1.2.4. Topoisomerase inhibitors	
2.1.2.5. Mitotic inhibitors	
2.1.3. Limitation of chemotherapy	
2.1.3.1. Lack of selectivity of anticancer drugs 10	
2.1.3.2. Multidrug resistance (MDR) 10	
2.1.4. Role of Plants as Medicinal and Anticancer Agents 10	

2.1.4.1. Plant derived Anticancer Agents in Clinical Use	11
2.1.4.1.1. Vinca Alkaloids	11
2.1.4.1.2. Podophyllotoxin Derivatives	12
2.1.4.1.3. Taxanes	12
2.1.4.1.4. Camptothecin Derivatives	13
2.1.4.1.5. Homoharringtonine	16
2.2. cancer chemoprevention	16
2.3. Role of free radicals in development of cancer	17
2.4. Antioxidants	18
2.5.Role of antioxidant in preventing cancer	18
2.3. Candida albicans and its relationship with cancer	19
2.4.Anticancer drug Discovery	20
4.1. Anticancer drug discovery from natural sources	21
2.4.1.1. Bioassays and Drug Discovery	22
2.4.1.1.1. Mechanism-based assays for anticancer drugs	22
2.4.1.1.2. Cell-based assays for anticancer drugs	23
2.5. History of 3D cancer spheroids <i>in vitro</i> model	24
2.5.1. Characteristics of spheroids	25
CHAPTER 3	
3. Material and Methods	
	•

3.1.1. Plant Material	28
3.1.2.Chemicals	32

Contents

3.1.3. Biological materials	33
3.2. METHODS	34
3.2.1. Collection of plant material	34
3.2.2. Preparation of plant extracts	34
3.2.3. Cell culture	34
3.2.4. Cell viability assay (Primary screening)	35
3.2.5. Determination of IC_{50} values (Secondary screening)	35
3.2.6. Selectivity Index (SI)	35
3.2.7. Generation of spheroids	36
3.2.8. Radical scavenging activity (DPPH method)	36
3.2.9. Determination of antioxidant EC ₅₀ Values	37
3.2.10. Anticandidal assay by using the disk diffusion technique	e.37
3.2.11. Initial fractionation	38
<u>CHAPTER 4</u>	
4- Results	
4.1. Anticancer activity	46

4.1. Anticalect activity	40
4.1. a. The most active extracts against lung cancer cell line (A549).	46
4.1. b. The most active extract against colon carcinoma cell line (HCT-116)	48
4.1. c. The most active extract against Hepatocellular carcinoma cell line (HepG2)	50
4.1.d. The most active extract against breast adenocarcinoma cel line (MCF-7)	1 52

Contents

4.1.1. Dose response study of bioactive crude extracts	54
4.1.1.a. Dose response study of the most active extracts against lung carcinoma cell line (A549)	56
4.1.1.b. Dose response study of the most active extracts against colon carcinoma cell line (HCT-116)	58
4.1.1.C. Dose response study of the most active extracts against hepatocellular carcinoma cell line (HepG2)	60
4.1.1.d. Dose response study of the most active extracts against breast adenocarcinoma cell line (MCF-7)	62
4.1.2. Selectivity index	64
4.1.3. In vitro cytotoxic effect on 3D (spheroids) cancer clles	66
4.1.3.a. Screening of the most active extracts which possessed l anticancer activity against Lung carcinoma cell line (A549) o	•
vitro 3D (spheroids) lung car	
vitro 3D (spheroids) lung car	ncer 67
vitro3D(spheroids)lungcarcelles	ncer 67 gh 70 gh
vitro3D(spheroids)lungcarcelles4.1.3.b. Screening of the most active extracts which showed hig activity against colon carcinoma cell line (HTC116) on <i>in vitro</i> 3D(spheroids) colon cancer celles4.1.3.C. Screening of the most active extracts which showed hig activity against Hepatocellular carcinoma cell line (HepG2) on	ncer 67 gh 70 gh <i>in</i> 73 nigh
vitro3D(spheroids)lungcarcelles	ncer 67 gh 70 gh <i>in</i> 73 nigh (F7)
vitro3D(spheroids)lungcatcelles	ncer 67 gh 70 gh <i>in</i> 73 nigh (F7) 76

4.4.1. The minimum inhibitory concentration (MIC) of the most active extracts
<u>CHAPTER 5</u>
5. Discussion
5.1. Anticancer activity 110
5.2. Antioxidant activity 113
5.3. Anticandidal activity 114
5.4. Conclusion
Summary 118
References
Arabic Summary

LIST OF TABLES

Table 1: Plant Material Collected for the Study	28
Table 2: Cytotoxicity of methanol plant extracts (100µg/ml) on four hum tumor cell lines: lung carcinoma (A549) and colon carcinoma (HCT-116), hepatocellular carcinoma (HepG2), breast carcinom (MCF-7)	L
Table (3): <i>In vitro</i> cytotoxic activity (IC ₅₀ μg/ml) of crude extracts tested against different cancer cell lines after24 hours	1 55
Table 4: The selectivity index (SI) values of the seven active plant extracts.	65
Table 5: Screening of the most active extracts on the A549 spheroids model.	68
Table 6: Screening of the most active extracts on the HCT-116 spheroids model.	71
Table 7: Screening of the most active extracts on the HepG2 spheroids model	74
Table 8: Screening of the most active extracts on the MCF-7 spheroids model.	77
Table 9: Free radical scavenging activity of methanol plant extracts by usDPPH assay at one concentration 100µg/ml	sing 92
Table 10: EC_{50} (the concentration required to scavenge 50% of the DPPH	I). 98
Table 11: In vitro activity of plant extracts against Candida albicans	101
Table 12: The minimum inhibitory concentration (MIC) of most active extracts.	108