بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

MONA MAGHRABY

Evaluation of Antitumor Activities of Extracts of non-edible parts of some plants common in Egypt: *in vitro* and *in vivo* Tests.

A thesis

submitted for the degree of Master of Science as a partial fulfillment for requirements of the Master of Science in Zoology

BY

Nermeen Mahmoud Mohmed Mahmoud
B.Sc., Zoology, Faculty of Science, Ain Shams University

Supervised by

Prof. Dr. Mohamed Abdelmordy Mohamed
Professor of Genetics and Molecular biology
Zoology Department-Faculty of Science
Ain Shams University

Prof. Dr. Khaled Mahmoud Mohamed Hanafi

Researcher professor of Biochemistry
pharmacognosy Department
National Research Centre

Zoology Department faculty of Science Ain Shams University

2020

Thesis Examination committee

Name **Signature**

Supervision

Dr. Mohamed Abdelmordy Mohamed
Professor of Genetics and Molecular biology
Zoology Department-Faculty of Science
Ain Shams University

Dr. Khaled Mahmoud Mohamed Hanafi
Researcher professor of Biochemistry
pharmacognosy Department
National Research Centre

2020

Approval Sheet

Evaluation of Antitumor Activities of Extracts of non-edible parts of some plants common in Egypt: *in vitro* and *in vivo* Tests.

By

Nermeen Mahmoud Mohmed Mahmoud

Submitted to

Faculty of Science, Ain Shams University

Supervision committee **Approved**

Prof. Dr. Mohamed Abdelmordy Mohamed
Professor of Genetics and Molecular biology
Zoology Department-Faculty of Science
Ain Shams University

Prof. Dr. Khaled Mahmoud Mohamed Hanafi

Researcher professor of Biochemistry
pharmacognosy Department
National Research Centre

Contents

Acknowledgment

First and foremost, praises and thanks to Allah, the Almighty, for his showers of blessings throughout my research work to complete the research successfully.

I would like to express my deep and sincere gratitude to my research supervisors: Prof. **Mohamed Abdelmordy Mohamed** and Prof. **Khaled Mahmoud Mohamed Hanafi.** However, I am profoundly indebted to my mentor Prof. **Mohamed Abdelmordy** for suggesting the subject and teaching me how to present the research work as clearly as possible. It was a great privilege and honour to work and study under his guidance. It worth mentioning that Prof **Khaled** who taught me how to practice cell culture techniques. I have been extremely lucky to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly.

I would like to extend my thanks to Assist. Prof. **Waleed Fayad**, Pharmacognosy Department, National Research Center, Dokki, for his dynamism, help, stimulating suggestions and continuous motivation all through the time of research. Also, I especially thank Prof. **Salwa El-Halowty**, Assist. Prof. **Mai El-Manwaty** and **Dr Ahmed Abdelfatah** Pharmacognosy

Department, National Research Center, Dokki for their help and time.

I would like to thank my family, especially my beloved father and mother, who made this arduous journey much more pleasant. Their love and helpful spirit had motivated me to achieve beyond my expectations.

Finally, I’m so grateful to my partner for his support in this period and the future life.

Abstract

Extracts of leaves, seeds, and peels of some vegetables and fruits were tested on cancer cells *In-vitro* and *in-vivo*. Leaves were obtained from **broccoli**, **beet**, and **turnip**. Seeds were from **Kurrat**, **parsley**, **spinach**, **chicory**, and **apricot**. Peels were from: **mango**, **pomegranate**, **golden berry**, **taro**, **okra**, **figs**, **tangerine**, **pea**, **avocado** and **banana**. The *In-vitro* studies were on monolayer human tumor cell lines including Liver carcinoma (**HEPG2**), Prostate adenocarcinoma (**PC3**), Breast adenocarcinoma (**MCF7**) and Colorectal carcinoma (**HCT116**) in addition to normal human epithelial cell line (**RPE1**). It also included 3D spherical models that were prepared from **MCF7**, **HCT116 and RPE1.**

The results of the **Primary Screening** showed that the **MCF7** were most affected by plant extracts, as their sensitivity was significantly high for nine extracts: **Golden berry, Kurrat, Avocado, Parsley, Mango, Pea, Spinach, Beet,** and **Chicory.** Next are colon cells (seven extracts), prostate (four), and finally liver cells (three extracts). In all cellular lines, extracts of **Avocado, Kurrat, and Golden berry** were on the top, where the percentages of dead cells (the cellular toxicity of these extracts) were high.

In **the Secondary Screening**, the $\mathbf{IC_{50}}$ was determined for each of the extracts that showed elevated cellular toxicity, (their number was ten) on the cancer cells. The results were as in the first **screening**, where the **MCF7** were the most sensitive to the extracts used. The values of the $\mathbf{IC_{50}}$ were low, and the golden berry extract was at the first (4.49 µg / ml), followed by avocado (6.43), then leek (41.11). The second most affected cell line was **HCT116** with values of 8.9 µg / ml for avocado, 23.7 for golden berry, and 40.6 for leek. In third place were the **PC3** cells with values of (8.3, 38.2, 43) for avocados, golden berry, and leek, respectively. Finally, the **HEPG2** cells were in fourth place with values: 12.5 µg / ml for avocado, 73 golden berries, and 79.3 for the leek. In the case of **RPE1** cell line, the cellular toxicity of the previous extracts was low, and its value ranged from 27.5% to 42.8%. While the cytotoxicity was between

68.04 - 99.8% in the case of the four cancer cells. This indicates that the normal cells are affected slightly.

The **spherical model** of colon cells was the most sensitive to the golden berry extract, then the breast model, and the normal cell model was not affected yet. The **IC_{50}** values were (19.2, 62, 124) respectively. The golden berry extract has destroyed the breast and colon models significantly, and the normal cell model has not been affected, which indicates that the golden berry extract has a high penetration force.

***In-vivo* studies:** Where a cancerous tumor was developed in mice by injecting them with **C26** cancer cells. The tumor was monitored day after day for five weeks. Some of tumor-bearing mice were treated with the golden berry extract, and before treatment the average tumor size was 155 mm^3 and began to decrease gradually after the treatment until it reached 18.9 mm^3. This indicates the positive effect of golden berry extract on reducing tumor size. By microscopic examination of sections in the tumor taken from mice untreated with the extract, and other sections in the tumor taken from mice treated with the extract, the cancerous cells were many, complete and spread among the degenerated muscle fibers in the first group. But in the second, the cancerous cells appeared with shrinked nuclei and most of them were dead. This also proves on the positive effect of golden berry extract on reducing tumor.

In Conclusion

In this research, it was possible to have extracts from leaves, seeds, and peels, from some plants common in Egypt. These extracts had effective positive results on cancer cells both *In-vitro* and *In-vivo* and were characterized by slight or no harmful effects on normal (healthy) cells. This is in contrast to the common chemotherapeutics that destroy cancer cells and have bad side effects on normal cells.

Therefore, expansion of this type of research should be encouraged in the hope of obtaining natural therapeutics for cancerous tumors, and these therapeutics are cheap, safe, and without harmful side effects.

Keywords: Non-edible, Cytotoxicity, **MCF7**, **HCT116**, **HEPG2**, **PC3, RPE1**, **3D** Spheroid,In-vivo studies.

List of abbreviations

Abbreviation	Meaning (Definition)
A549	Human lung, Carcinoma,
AFB1	aflatoxin B1
APC	adenomatous polyposis coli
ATCC	American Type Culture Collection
B16F10	*Mus musculus*, mouse Melanoma
BALB/c	is an albino, laboratory-bred strain of the house mouse from which a number of common substrains are derived. Now over 200 generations from New York in 1920,
Bax	BCL (B Cell Lymphoma)-Associated X
BCL2	B-cell lymphoma 2
BRCA1	BReast CAncer gene 1H
BRCA2	BReast CAncer gene 2
C26	Colonic Adenocarcinoma 26
CACO-2	heterogeneous human epithelial colorectal adenocarcinoma cells
caspase 3	cysteine-aspatric proteases 3
caspase-7	cysteine-aspatric proteases 7
CCl_4	Carbon tetrachlorid
CPT	Camptothecin-11
CRC	Colorectal cancer
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediamine tetraacetic acid
ER	The estrogen receptor
FBS	fetal bovin serum
GLOBOCAN	Global cancer obsrvatory
GPCR	The Gharbiah Population-based Cancer Registry
GWAS	Genomewide association studies
GWAS	Genome wide association studies
HA22T	hepatoma cells
HBV	hepatitis B virus
HCC	Hepatocellular carcinoma
HCT116	Colorectal carcinoma
HCV	hepatitis C virus
HDACs	Histone deacetylases
Hela	
HEP3B	heptocellular carcinoma

HEPG2	Liver carcinoma
HER2	human epidermal growth factor 2
HGF1	human normal gingival fibroblast
HI99	lung; derived from metastatic site: lymph node
HPV	human papilloma virus
HT	high- throughput
HT-29	Human colonic ADENOCARCINOMA cells that can express differentiation features characteristic of mature intestinal cells such as the GOBLET CELLS
HTCL	Human tumour cell line
HTS	high-throughput screening
IARC	International Agency for Research on Cancer
IC_{50}	half maximal inhibitory concentration
JMAR and MDA-1986	head and neck squamous cell carcinoma cell lines
LD_{50}	median lethal dose
MCF7	Brest adenocarcinoma
MCS	Multicellular Spheroid
MDA-MB-231	Human adenocarcinoma, mammary gland/breast; derived from metastatic site
MECC	the Middle East Cancer Consortium
MIDA-MB231	breast cancer cell line
MRC-5	normal fetal lung fibroblast
MTT	3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyl tetrazolium bromide
NCI	The National Cancer Institute
NCRPE	The National Cancer Registry Program of Egypt
OECD	The Organisation for Economic Co-operation and Development
PBS	phosphate buffer solution
PC3	Prostate adenocarcinoma
PCa	Prostate cancer
PCF	Prostate cancer foundation
PLC/PRF15	BALB/C normal liver cells
PNPP	para-Nitrophenylphosphate
PR	The progesterone receptor
PTEN/MMAC1	phosphatase, tensin homologue/mutated in multiple advanced cancers
QOL	Quality of life
RPE1	normal human epithelial cell line

SBSC	Schistosome Biological Supply Centre
SDS	Sodium dodecyl sulfate
SKMEL-28	Human Melanoma
SNPs	Single nucleotide polymorphism
TBRI	Theodore Bilharz Research Institute
TP53	Tumor Protein 53
US FDA	United States Food and Drug Administration
US FDA	United States Food and Drug Administration
USA	United states of America
WHO	World Health Organization