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Anti-tumor and Antimicrobial Activity of Nicotine Extracted from *Nicotiana tabacum* L. and its Effect on Cell Cycle Genes Expression

A Thesis

Submitted to The Council of The College of Science - University of Diyala as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

By

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Committee Certification

We, as the examining committee, certify that we have read this thesis and its contents and examined the student (**Amenah Khudhair Khalaf**), found it adequate as a thesis for the Master's Degree of Science in Biology.

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I, certify that this thesis entitled **(Effect of Alkaloids Nicotine from Tobacco in Gene Expression on Cell Line)** has been conducted under my supervision at the College of Science, University of Diyala, as partial fulfillment of the requirements for the Master Degree of Science in Biology .

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Given the available recommendation, I forward this thesis for debate by the examining committee.

Signature

Dedication

I dedicate this work to ….

My dear parents, whose love, encouragement, support, and prays of days and nights make me able to get such success and honor.

My sisters are always standing by my side.

Amenah

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Summary

Nicotiana tabacum L. is an annual herbaceous plant, containing a diversity of bioactive secondary metabolites such as nicotine which have anticancer and antimicrobial activities. Cancer is a major health problem and an important cause of death worldwide. Bacterial and fungal infections are a big problem in medicine due to their opportunistic properties.

This study aimed to determine the antimicrobial, anti-tumor, and cytotoxicity of nicotine extracted from Tobacco (*Nicotiana tabacum* L.) and their effect on some cellular genes expression.

Dragendroff's reagent and Gas Chromatography\Mass Spectroscopy method is used to detect the level of nicotine in the extract. The bacterial species used in this study include *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, and the yeasts species include *Candida albicans* and *Rhodotorula mucilaginosa*. The extract's antibacterial and antifungal activities were detected by using the agar well diffusion method.

The cell lines used in this study include (Michigan Cancer Foundation-7) human breast cancer, (Henrietta lacks) human cervical cancer cell lines, and (Rhabdomyosarcoma) human muscle tissue cancer cell lines. These lines were all subjected to various extract concentration treatments. The gene expression of Heat shock protein70, Hypoxia-inducing factor, B-cell lymphoma-2-Associated-X-protein, P53, and Caspase-9 were had been analyzing by quantitative Real-Time Polymerase Chain Reaction by using a stain of Kapa syber green, and the morphological changes were observed by using a stain of Acridine Orange/ Propidium Iodide and inspected by using a fluorescence microscope.

In results show that the higher inhibition zone was observed in *Staphylococcus aureus* and *Candida albicans*, which was 30.3 mm and 30.0 mm respectively, followed by *Pseudomonas aeruginosa*, *Escherichia coli*, *Rhodotorula mucilaginosa*, and *Acinetobacter baumannii* at the diameter of its inhibition (27.7, 19.7, 19.3 and 13.7) respectively.

The inhibitory effects of the extract on the growth of (Michigan Cancer Foundation-7) were 20.07% at (50 µg/ml), but it increased to 96.67% at (800 µg/ml), and the inhibition rate on (Henrietta lacks) was 34.87% at (50 µg/ml) and the rate increased to 90.31% at (800 µg/ml). While in the Rhabdomyosarcoma cell line, there is no significant inhibition at (50 µg/ml) but it is increased to 15.63% at (800 µg/ml). Also, treating cells with the extract at IC_{50} suppresses the gene expression of heat shock proteins70 and Hypoxia-inducing factor in Michigan Cancer Foundation-7 which was (0.42 and 0.14) respectively, and in Henrietta lacks was (0.10 and 0.76) respectively, while increasing the gene expression of apoptosis encoding genes which include P53 in three cell lines which was (0.13, 0.17 and 1.68), and of B-cell-lymphoma-2-Associated-X-protein was (0.47, 0.53 and 0.42), while the gene expression of Caspase9 was (1.90, 2.69 and 3.33). In addition, numerous morphological changes were observed in apoptotic cells including DNA fragmentation, chromatin condensation, cell shrinkage, membrane blebbing, and apoptotic body formation.

The study concluded that the nicotine extract of *Nicotiana tabacum L.* has antimicrobial and anti-tumor effects, and it can induce apoptosis and inhibit the gene expression of heat shock proteins encoding genes in cancer cells making this plant a promising option for cancer and microbial infection treatment but is needed for more research.

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List of Abbreviations

Abbreviate	Meaning
A375	Human Melanoma Cell Line
A549	Adenocarcinoma Human Alveolar Basal Epithelial Cells
AMJ13	Invasive Ductal Carcinoma
AO\PI	Acridine Orange\Propidium Iodide Stain
Apaf-1	Apoptotic Protease Activating Factor-1
ApE	A Plasmid Editor
ATP	Adenosine Triphosphate
BAD	Bcl-2 Associated Agonist of Cell Death
BAK	Bcl-2 Antagonist Killer
BAX	Bcl-2 Associated X-Protein
Bcl-2	B-Cell Lymphoma 2
BID	BH3 Interacting Domain Death Agonist
BLAST	Basic Local Alignment Search Tool
CBDs	Cembranoid-type Diterpenes
cdk2	Cyclin-Dependent Kinase 2
C-myc	Cellular-Myelocytomatosis
E2F	Expression Factor
ECM	Extra-Cellular Matrix
EDTA	Ethylene Diamine Tetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent assay
Erb-B-1	Epidermal Growth Factor Receptor
FCS	Fetal Calf Serum
FDO	Food and Drug Organization
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GC/MS	Gas Chromatography/Mass Spectrometry
H22	Hepatoma-22 Cell Line
HCT116	Human Colorectal Carcinoma Cell Line
Hela	(Henrietta Lacks) Cervical Carcinoma
HepG2	Liver Hepatocellular Carcinoma Cell Line
HIF	Hypoxia-Inducing Factor
H-ras	Harvey Rat Sarcoma Virus
HRT-18	Human Rectal Tumor-18 Cell Line
HSEs	Heat-Shock Elements
HSF1	Heat-Shock Factor 1
Hsps	Heat Shock Proteins

HT-29	Human Colorectal Adenocarcinoma Cell Line
IC_{50}	Inhibiting Concentration $_{50}$
ICCMGR	Iraqi Center for Cancer and Medical Genetics Research
LNCaP	Lymph Node Carcinoma of the Prostate
LPS	lipopolysaccharide
MCF7	Michigan Cancer Foundation-7
MDA-MB-231	MD Anderson-Metastatic Breast-231 Cell Line
miRNAs	Micro Ribonucleic Acid
M-MLV	Murine Leukemia Virus
NCBI	National Center for Biotechnology Information
NCI-H292	Non-Small-Cell Lung Cancer Cell Line
N-myc	Neuroblastomas- Myelocytomatosis
OGs	Oncogenes
OmpA	Outer Membrane Protein A
PBS	Phosphate Buffer Saline
PC3	Prostate Carcinoma 3
PHA	Phytohaemagglutinin
PK	Proteinase K (Proteinase Keratin)
Ramp	Reliability Analysis and Modeling Program
RB	Retinoblastoma
RD (RMS)	Rhabdomyosarcoma
Rf value	Retardation Factor Value
ROS	Reactive Oxygen Species
rpm	Revolution Per Minutes
RPMI-1640	Roswell Park Memorial Institute-1640
SDS	Sodium Dodecyl Sulfate
siRNA	Small Interfering Ribonucleic Acid
Sis	Platelet-Derived Growth Factor (PDGF)
SK-GT2	Gastric Fundus Carcinoma
SKGT-4	Esophagus Cancer Cell Line
TAE Buffer	Tris-Acetate-EDTA Buffer
TSGs	Tumor Suppressor Genes
U251-MG	Brain Cancer Cell Line
WHO	World Health Organization
WI-38	Human Lung Tissue Cell Line

Chapter One
Introduction

1.1 Introduction

Nicotiana tabacum L.(*N. tabacum L.*) is an annual herbaceous plant, containing a diversity of biologically active secondary metabolites (Ramesh and Valan, 2018; Shang *et al*., 2019). The therapeutic properties result from different parts of this plant, due to the presence of low-molecular-mass secondary metabolites such as phenolics, terpenoids, flavonoids, and alkaloids (Kuruppu *et al.*, 2019).

Cancer is one of the major health problems in the world and is one of the important causes of an increase in deaths among children and adults. According to the WHO, After cardiovascular disorders, cancer is the second most prevalent cause of mortality worldwide (Krupa-Kotara and Dakowska, 2021), as the number of people living with cancer reached 21.2 million people in 2021 (Sung *et al.*, 2021; Siegel *et al.*, 2022). Breast cancer is the most commonly diagnosed cancer and is followed by lung cancer, then colorectal cancer, prostate cancer, non-melanoma of skin cancer, and stomach cancer (Ferlay *et al.*, 2021). Although surgical, radiation and pharmaceutical such as chemotherapy, gene therapy, hormone therapy and immunotherapy are traditional methods of cancer treatment, they didn't achieve the required results and they have many side effects (Schirrmacher, 2019; Zhang *et al.*, 2020; Zhang *et al*., 2021).

Bacterial infections are a big problem in medicine, and one of the main reasons is that a large percentage of these infections are endogenous, meaning that the etiologic agents come from the human bacterial flora (Kolář, 2022). A fungal infection is an opportunistic infection that can be fatal, and in recent years, it has become more common. This infection can have an impact on a patient's prognosis as well as increase costs for patients and their families (Wen *et al.*, 2022). Antibiotics have had a great effect in

fighting microbial infections and benefited the health-related quality of human life since their introduction. However, these health benefits have been compromised in recent decades because many widely used antibiotics have been less effective against some infections, not only because many of them can cause toxic reactions, but also due to the emergence of drug-resistant microbes (Bhalodia and Shukla, 2011).

1.2 Aims of the Study

This study aims to determine the antimicrobial, anti-tumor, and cytotoxicity of nicotine extracted from Tobacco (*Nicotiana tabacum* L.) and its effect on some cellular genes expression.