EVALUATION OF THE ACTIVITY OF SOME FRUIT WASTES AGAINST VIRUSES AND CANCER CELL LINES

Submitted By

Eman Amin Esmail Mohamed

B.Sc. of Agricultural Sciences, Faculty of Agriculture, Cairo University, 1990

A thesis submitted in Partial Fulfillment
Of
The Requirement for the Master Degree
In
Environmental Sciences

Department of Environmental Agricultural Sciences
Institute of Environmental Studies and Research
Ain Shams University

2016

APPROVAL SHEET

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Eman Amin Esmail

Abstract

Eman Amin Esmail Mohamed: "Evaluation of the activity of some fruit wastes against viruses and cancer cell lines".Unpublished Master of microbiology. Department of Agriculture Science Institute of Environmental Studies and Research .Ain Shams University 2016.

The present work aimed to evaluate the antiviral and anticancer potentials of reusable orange albedo extract (OAlbE) and grape seed extract (GSE) wastes. Results revealed that the **IC_{50}**values of OAlb M/E and GSE M/E extracts with MCF-7 cell lineswere 184439, 48108, 344,11.68 µgm / ml and was 6016 µgm / ml for SGSE 24 hrs post treatment respectively while the **IC_{50}**values of the extracts with Caco-2 cell lines were 23708, 14107, 1158,15607 µgm / ml and was 430 µgm / ml for SGSE 24 hrs post treatment, respectively . The SGSE was of a higher potential toxicity to experimental extracted one. Antiviral activity of E/M OAlbE and GSE showed the same antiviral potential against RVFV recording 0.51 $\log_{(10)}$ / 0.1 ml ; (6.58%) and 1 $\log_{(10)}$ / 0.1 ml (12.9%) respectively. While the antiviral potential against HAV revealed that OAlbE/M and GSME showed low potential against HAV recording 0.26 $\log_{(10)}$ /0.1 ml. On the contrary both O-AlbE and GSME showed no antiviral potentials compared with Standard IFN (5 IU/ml) recorded a depletion of viral infectivity titers recording 3.25 $\log_{(10)}$ / 0.1 ml (41.93%) and 3 $\log_{(10)}$ /0.1 ml (40%) for RVFV and HAV respectively. Also, anticancer potentials was proved as there was a remarkable *BCL-2 , P53* and *Bax* genes expression compared with control respectively. It can be concluded

that both E/M OAlb and GSE are promising antiviral and anticancer agents. More intensified investigations must be conducted to maximize the biological potentials of both extracts. A higher level of characterization of extract contents must be achieved and evaluated for targeting the most promising fraction has both bioactivities considered .

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List of abbreviations

AIF	Apoptosis inducing factor
ATCC	American Type Culture Collection
Bax	Bcl-2–associated X protein
Bcl-2	B-cell lymphoma 2
Bp	Base pair
Caco2	Colon cancer cell
CAD	Caspase-activated Dnase
CAPs	Cationic antimicrobial peptides
$CCID_{50}$	Cell culture infectious dose 50%
cDNA	Complementary DNA
CPE	Cytopathogenic (cytopathic) effect
DISC	Death-inducing signaling complex
DEPC	Diethyl pyrocarbonate
Df	Dilution factor
DMSO	Di-methyl sulfoxide
EAC	Earlish cancer cell
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
EMEM	Earle's minimum essential medium
Endo G	Endonuclease G
FAAD	Fas-associated death domain
FBS	Fetal bovine serum
GrB	Granzyme B
GSE	Grape seed ethanol extract
GSM	Grape seed methanol extract
GSS	Grape seed standard
HAV	Hepatitis A virus
IAP	Inhibitors of apoptosis proteins
IC_{50}	Inhibitory concentration $_{50}$

KDa	Kilodalton
LDH	lactate dehydrogenase enzymes
MCF-7	Human breast carcinoma cells (HTB-22)
MICs	Minimum inhibitory concentrations
MOI	Multiplicity of infection
MTT	3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyl tetrazolium bromide
OAE	Orange Albedo ethanol extract
OAM	Orange Albedo methanol extract
OD	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
PS	Phosphatidylserine
ROS	Reactive oxygen species
Rpm	Round per minute
RT-PCR	Reverse transcription-polymerase chain reaction
RVFV	Rift valley fever virus
SDS PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SD	Standard deviation
TAE	Tris Acetate EDTA
TNF	Tumor necrosis factor

Introduction

1-INTRODUCTION

Fruits and vegetables wastes and by-products, which are formed in great amounts during industrial processing, represented a serious problem, as they exert an influence on environment and need to be managed and/or utilized. On the other hand, they are very rich in bioactive components, which are considered to have a beneficial effect on health. Using the agro wastes therapeutically are new ideas which are slowly gaining popularity. They are high value products and their recovery will be economically attractive. These are novel, natural, eco friendly and economic sources of antimicrobics, which can be used in the prevention of diseases caused by pathogenic microbes and also reduce pollution (**Chanda , *et al*., 2010**). Citrus fruit are consumed as fresh or utilized for processed citrus products and citrus-by-products. The peel of citrus fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants. *In vitro*, flavonoids display anti-proliferative effect on various human neoplasic cell lines as observed in myeloid and lymphoid leukemia cells (**Larcocca , *et al* .,1990)** gastric, ovarian, Prostrate cancer cells (**Peterson and Barnes., 1993**), and squamous cell carcinoma (**Kandaswami , *et al* ., 1991**). Another working group proved that, the antioxidant and antimicrobial properties of methanol (100% and 80% aqueous) extracts pummelo fruit's Albedo (*Citrus grandis* Osbeck), contain by-products responsible components were purified, and the isolated compounds were tested for antioxidant and antimicrobial potential **(Mokbel and Suganuma ,2006)**.Vitis vinifera (grape) is a rich source of several biologically active compounds including anthocyanins, proanthocyanidins, and stilbenes (**Asl and Hosseinzadeh ,2009**). Grape seed extract (GSE), a mixture