BIOCHEMICAL CONSEQUENCES OF SOME ACTIVE INGREDIENTS ISOLATED FROM *Annona* SPECIES GROWN IN EGYPT THROUGH GROWTH SEASON

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2006
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APPROVAL SHEET

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Name of Candidate: Mona Arafa Mohammed Ibrahim **Degree:** Ph.D
Title of Thesis: Biochemical Consequences Of Some Active Ingredients Isolated From *Annona* Species Grown in Egypt Through Growth Season.
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Department: Agricultural Biochemistry **Approval: 2 /11/ 2016**

ABSTRACT

The study investigated PCR based DNA fingerprinting in a set of three species of *Annona* L. (*Annona squamosa*, *Annona cherimola* and *Annona* Abdel Razek; the hybrid between them) using RAPDs (5 primers) and ISSRs (5 primers). The RAPD and ISSR markers were analyzed using SPSS program and the results were presented in two dendrograms to address the relationships among the samples studied. The three *Annona species* were screened for the presence of major phytochemical compounds. *Anonna* extracts showed the presence of flavonoids, carbohydrates, tannins, triterpenoids, steroids, and alkaloids as major groups. Qualitative and quantitative analyses of volatile oil of three species were performed.

In addition, structural analysis *via* HPLC-ESI-MS^n, UPLC-HESI-MS/MS and NMR reported 65 secondary metabolites in the three *Annona species*. Seven flavonoids are identified as Rutin, Kaempferol 3-glucoside-7-rhamnoside, Astragalin, Kaempferol, Quercetin, Hirsutrin and Luteolin-7-O-β-D-glucopyranoside. Five alkaloids are identified as Liriodenine, Lanuginosine, Oxostephanosine, Stepharine, and Coclaurine. The biological evaluations of the three species took place through the determination of their *in vitro* antioxidant effect. The more pronounced extracts were *in vivo* investigated as anti-gastroulcerative agent in rats. The stomach histopathological study was done for results confirmation. In conclusion, *Annona cherimola* and the hybrid extract recorded the most *in vitro* antioxidant effect and served as anti-gastroulcerative agents.

The different extracts of the three *Annona* species were screened for their effect on DPPH and $ABTS^{\cdot+}$ to determine their free radical scavenging. The $ABTS^{\cdot+}$ IC_{50} for Abdel Aazek and *A. cherimola* bark are 9.2 and 28.62 and more active than the standard drug respectively.

Volatile oils are the best extracts by using HTC116, PC3, HepG2, MCF7 (cancer cell) and RPE1 (normal cell) and may be important economically inexpensive cancer treatment.

Key words: *Annona spp*., Flavonoids, Acetogenins, Phenolics, Alkaloids, Antioxidant, DPPH, $ABTS^{\cdot+}$, Anti-gastroulcerative Agent, Histopathology study, Cytotoxicity.

DEDICATION

I dedicate this work to whom my heart felt thanks; to my soul Mother and Father, as well as to my brothers; Mohammed and Shreef, my sister; Nermeen and Dr. Souad El-gengaihi for all the support their lovely offered along the period of my post graduation.

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ABBREVIATIONS

5'NT	5'-Nucleotidase
$ABTS^{\bullet+}$	2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) anion radical
ACGs	Annonaceous acetogenins
AGC	automatic gain control
AIF	All Ion Fragmentation
AK	Actinic Keratosis
$AlCl_3$	Aluminium chloride
AMPG	aqueous methanolic extract of *Punica granatum*
AP	Acid Phosphatase
BA	Bark Abdel Razek
BAW	Butanol : Acetic acid: Water
BB	Bark *Annona squamosa*
Bcl-2	Beta cell leukemia lymphoma
BDH	orthophosphoric acid
BH	Bark *Annona cherimola*
bp	Base peak
BSA	Bovine serum albumin
BuOH	Butanol
C18	Column C_{18}
CAT	Catalase
$CDCL_3$	Deuterated Chloroform
CEAD	Colometric electrode array detector
CEO	*Citrus aurantium* essential oil
Ch	Cholinergic
COX	cyclooxygenase
CPA	centipedic acid
d	Doublet
d.w	Diionized water
ddH2O	bi-distilled water
DHBS	3,5-dichloro-2-hydroxy-benzene sulfonic acid
DMEM	Dulbeco's Modified Eagle's Medium
DMSO	Di Methyl Sulphoxide
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide nucleoside triphosphate
Dp	Degree of polymerization
$DPPH^{\bullet}$	1,1-diphenyl-2-picrylhydrazyl radical

DTNB	5,5'-dithiobis (2-nitrobenzoic acid)
Dw	Dry weight
EBV-EA	Epstein-Barr Virus Early Antigen
ED_{50}	Effective Dose 50
EI	Electron Ionization
ER	Endoplasimic Reticulim
ESI	Electrospray inozation
ESI-MS^n	electrospray ionization- mass spectrometry
EtOAc	Ethyl acetate
EtOH	Ethanol
FA	Fruit Abdel Razek
FAD	Flavin adenine dinucleotide
FB	Fruit *Annona squamosa*
FBS	Fetal Bovine Serum
FC	Folin-Ciocalteu
FH	Fruit *Annona cherimola*
FID	Flam ionization detector
G6P	Glucose 6-phosphate
GAE	Gallic acid equivalents
GC	Gas chromatography
GC-MS	Gas chromatography - mass spectrometry
GERD	gastroesophageal reflux disease
GF_{254}	Silica gel, GF254, for thin layer chromatography
GG	guar gum
GI	Gastrointestinal
GLC	Gas liquid chromatography
GRIN	Germplasm Resources Information Network
GSH	Glutathione
H. pylori	*Helicobacter pylori*
H^+/K^+–ATPase	proton pump
H_2O_2	Hydrogen peroxide
HCD	Higher energy collision dissociation
HCT-116	Colon Carcinoma
$HepG_2$	human hepatocellular carcinoma cell line
Hex	Hexane
HOAc	Acetic acid
HPLC	High Performance Liquid Chromatography
HR-MS	High-resolution Mass Spectrometry
Hz	Hertz