# BIOCONVERSION OF SOME NATURALLY OCCURRING PHENOLIC AND TERPENOID COMPOUNDS

**Thesis**

**Presented By**


## Ehab Ghareeb Mohammed


**Submitted in partial fulfillment of the requirements of the Master Degree in Pharmaceutical Sciences (Pharmacognosy)**

**Under The Supervision of**


## Prof. Dr. Mohammed Hosny Hussein

**Prof. of Pharmacognosy, Faculty of Pharmacy "Boys"**
**Al-Azhar University, Cairo, Egypt.**

**Dr. Ehab Mohammed Moustafa**
**Lecturer of Pharmacognosy**
**Faculty of Pharmacy "Boys"**
**Al-Azhar University, Cairo, Egypt.**

**Dr. Mahmoud M. El-aasser**
**Lecturer of Microbiology,**
**Regional center of Mycology**
**Al-Azhar University, Cairo, Egypt.**

**Dr. Hassan Abdel Zaher Mohammed**

**Assoc. Prof. of Natural & Microbial chemistry**
**National Research Center**
**Cairo, Egypt.**

**Department of Pharmacognosy**
**Faculty of Pharmacy**
**Al-Azhar University**
**Cairo, Egypt.**


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**قـال الله تعالـي:**

بســم الله الرحمــن الرحيــم

**﴿ وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا ﴾**

**صــدق الله العظيــم (سورة النســاء – 113)**

Al-Azhar University
Faculty of Pharmacy
Pharmacognosy Department

# Certificate of Approval

This is to Certify that Master Thesis of

**Ehab Ghareeb Mohammed**

Has Been Approved By the Examination Committee

## Thesis Committee

Prof. Dr. Hazem Ahmed Kadry .....

Prof. Dr. Abd El-Naser Badawy Singab .....

Prof. Dr. Mohammed Hosny Hussein .....

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# DECLARATION

I hereby declare that the thesis entitled:

**"BIOCONVERSION OF SOME NATURALLY OCCURRING PHENOLIC AND TERPENOID COMPOUNDS"**

Submitted to Al-Azhar University, Faculty of Pharmacy, Pharmacognosy Department for

obtaining the degree of Master in Pharmaceutical Sciences (Pharmacognosy) as the result of the research work carried out by me at laboratories of Pharmacognosy Department, Faculty of Pharmacy and Regional center of Mycology, Al-Azhar University, Cairo, Egypt under the guidance of Prof. Mohammed Hosny Hussein, Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, during the period 2010-2013. I further declare that the results are not submitted for the award of any other degree or fellowship.

**Pharmacist: Ehab Ghareeb Mohammed**

**Assistant Researcher**

Al-Azhar University,

Faculty of Pharmacy,

Pharmacognosy Department

Cairo, Egypt.

# CERTIFICATE

I hereby certify that the thesis entitled

**“‘BIOCONVERSION OF SOME NATURALLY OCCURRING PHENOLIC AND TERPENOID COMPOUNDS”**

Submitted by Pharmacist: Ehab Ghareeb Mohammed for the degree of Master in Pharmaceutical Sciences (Pharmacognosy) to Al-Azhar University, Faculty of Pharmacy, Pharmacognosy Department. The research work was carried out by him at laboratories of Pharmacognosy Department and Regional center of Mycology, Cairo, Egypt, under my guidance and supervision during the period 2010 – 2013.

**Prof. Mohammed Hosny Hussein**

Pharmacognosy Department
Faculty of Pharmacy,
Al-Azhar University,
Cairo, Egypt.

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# List of Abbreviations

| | |
|---|---|
| **UV** | Ultraviolet |
| **IR** | Infra- red |
| **MS** | Mass Spectrometry |
| **ESI-MS** | Electro-spray Ionization Mass Spectrometry |
| ***m/z*** | Mass to Charge ratio |
| **$^{1}$H NMR** | Proton Nuclear Magnetic Resonance |
| **$^{13}$C NMR** | Carbon 13 Nuclear Magnetic Resonance |
| **DEPT** | Distortionless Enhancement by Polarization transfer |
| **HMQC** | Hetro-nuclear Multiple Quantum Coherence |
| **Hz** | Hertz |
| **MHz** | Mega hertz |
| **ppm** | Parts Per Million |
| **s** | Singlet |
| **d** | Doublet |
| **t** | Triplet |
| **m** | Multiplet |
| **brs** | Broad singlet |
| ***J*** | Coupling constant |
| **δ** | Chemical shift |
| **nm** | Nanometer |
| **DPPH** | 2,2–diphenyl–1–picrylhydrazyl |
| **BHT** | Butylated hydroxyl toluene |
| **MTT** | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| **$ED_{50}$** | Effective dose |
| **$IC_{50}$** | The half maximal inhibitory concentration |

# Abstract

Biotransformation is a fundamentally practical discipline in which the objective is to use enzymes and whole cells as catalysts for conversions of organic substrates. This subject is growing very rapidly, finding ever increasing applications ranging from pharmaceuticals and fine chemicals to food additives, cosmetic ingredients and biofuels. A biotransformation, as understood by the growing community of chemists and bio-scientists who practise in the area, is the conversion of one chemical entity to another by the action of a biological system which, in our case will be primarily micro-organisms or enzymes derived from them. Biotransformation has become an important tool in the area of synthetic organic chemistry and its application is continuously growing in pharmaceutical and chemical industries. It can provide a method of performing chemical reactions on an inaccessible site of organic compounds in a *regio*- and *stereo*-selective manner to generate derivatives that may be difficult to obtain by traditional organic synthesis.

Natural products are an incredibly diverse group of small (usually molecular weight less than 1500 Da) organic compounds isolated from a variety of natural sources, principally plants. The reason that natural products capture the imagination of organic chemists and pharmaceutical scientists is because of their well-documented and wide ranging biological activities and their skeletal diversity and intriguing functional group characteristics, which render them as indispensable leads for probing biological system status and for drug discovery with new bioassay systems. Biotransformations of several phenolic and terpenoid natural products are usually carried out by whole cells of bacteria, cyanobacteria, yeasts, microalgae, fungi, and plants or isolated enzymes. Nearly two-thirds of the papers published on the biotransformation of phenolic and terpenoid natural products are based on whole-cell transformations, with the majority of studies on bacterial and fungal transformations, while isolated enzymatic transformations are only responsible for 7% of the publications.

Research on the utility of microorganisms as models for mammalian drug metabolism has received considerable attention. The zygomycete fungi such as *Cunninghamella* and *Mucor* spp.have demonstrated the ability to metabolize xenobiotic compounds including pharmaceutical drugs and to produce drug metabolites that are known to be also formed in mammals.

In order to use fungal model to predict a mammalian drug metabolic pathway, we report, the biotransformation results of two phenolics (curcumin and aesculin) and a sesquiterpene lactone (α-santonin) using 16 different microorganisms.

Of the organisms which effected transformation, scale up studies was carried out with selected cultures (*Cunninghamella echinulata* (RCMB 012002) and *Mucor rouxii* (RCMB 015004) as biocatalyst according to the standard two-stage fermentation protocol to isolate the maximum number of metabolites detected in the culture broth in reasonable yields. We describe the isolation and structural elucidation of seven microbial metabolites (three metabolites from curcumin, one major metabolite from aesculin and three metabolites from α-santonin) produced by *Cunninghamella echinulata* and *Mucor rouxii*. The isolated metabolites were evaluated for their possible impact on the antioxidant and cytotoxic activities in relation to structures are also discussed.

The study includes a general introduction and the experimental techniques arranged in four different chapters.

## Chapter I

This is an introductory chapter and describes the literature related to various terminologies such as microbial transformation, chemistry and bioconversion of curcumin, aesculin and α-santonin and also about the current status of research on different pharmacological aspects of these compounds.

## Chapter II

This chapter gives details of experimental methods used in isolation of curcumin, aesculin and α-santonin metabolites with selected cultures (*Cunninghamella echinulata* (RCMB 012002) and *Mucor rouxii* (RCMB 015004). This chapter also gives details of experimental methods of biological evaluation (antioxidant and cytotoxicity) of the substrates and their isolated metabolites using different model systems.