Ain Shams University
Faculty of Pharmacy
Microbiology and Immunology Department

A Study on Microbial Biotransformation of Caffeic Acid by Some *Candida* species

A Thesis

Submitted for Partial Fulfillment of the Requirements for the

Master's Degree

In Pharmaceutical Sciences

(Microbiology and Immunology)

By

Raghda Abdel Nasser Badawi Singab

Bachelor of Pharmaceutical Sciences, 2013

Demonstrator, Department of Microbiology and Immunology

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والحمد لله رب العالمين

Raghda Abdel Nasser Singab

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List of Abbreviations

Abbreviation	Definition
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BLAST	Basic Logic Alignment Search Tool
CD_{50}	Cytotoxic dose 50
CLSI	Clinical and Laboratory Standard Institute
Cox-B4	Coxsackie virus type B4
CPE	Cytopathic effect
CV	Coefficient of variation
DDD	Double distilled deionized
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethyleneiaminetetraacetic acid
EMEM	Eagle's minimum essential medium
FBS	Fetal bovine serum
GMB	Glucose methylene blue
HAV-H10	Hepatitis A virus type H-10
HCV	Hepatitis C virus
HDAC	Histon deacetylase
HIV	Human Immunodeifiency Virus
HSV-1	Herpes Simplex virus type 1
Kgy	Kilo Gray
LC-ESI-MS	Liquid chromatography coupled with electrospray ionization mass spectroscopy
MEM	Minimum essential medium
MNTC	Maximum Nontoxic Concentration

MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
NCBI	National Centre for Biotechnology Information
OD	Optical density
PAL	Phenylalanine ammonia-lyase
PBS	Phosphate buffered saline
PFU	Plaque forming unit
ppm	Parts per million
RPMI	Roswell Park Memorial Institute
RSM	Response surface methodology
SDA	Sabouraud's dextrose agar
$TCID_{50}$	Tissue culture infectious dose 50
TLC	Thin Layer chromatography
UPLC	Ultra performance liquid chromatography
v/v	Volume/volume
w/v	Weight/volume

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Abstract

A total number of 92 clinical isolates of *Candida* species was utilized to perform caffeic acid biotransformation using two stage fermentation procedure. After 5 days of incubation, the cell free supernatants were collected and extracted using ethylacetate: n-butanol (9:1).

The success of biotransformation process was checked by TLC autography method and confirmed by nuclear magnetic resonance. The obtained results showed that 7 isolates could perform caffeic acid biotransformation.

The biological activities (antioxidant, antibacterial, antifungal, antiviral, and cytotoxic activities) of the obtained extracts were determined. According to the obtained results, the extract of isolate CI-24 had the most promising biotransformation ability. It had potential antibacterial activity against *Staphylococcus aureus* in addition to promising cytotoxic activity against Caco-2 cell line. As determined by LC-MS, caffeic acid was transformed by isolate CI-24 into para-hydroxy benzoic acid.

The isolate was genetically identified as *Candida albicans* (accession number MH356583) using 28S rRNA gene sequencing. The biotransformation ability of *C. albicans* CI-24 was improved through mutation by Gamma irradiation. Treatment with a dose of 5 Kilo Gray (KGy) resulted in 99.99% kill as determined by counting the survivors. The screening experiments showed that one mutant showed about 2.8 fold increase in para-hydroxybenzoic acid concentration when compared to the wild type.

In addition, response surface methodology experimental design (RSM) was used for optimization of physiological (nitrogen source) and environmental (temperature, initial pH, and agitation) factors. The main goal of applying RSM was to pinpoint efficiently for the optimum values of the process parameters to maximize the biotransformation process. The maximum para-hydroxybenzoic acid concentration (7.47 mg/ml) was obtained in a fermentation medium