Honey Induced Expression of Glutathione-Encoding Genes (*gsh*A and *gsh*B) in Clinical Isolates of *Pseudomonas aeruginosa*

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Certification

This is to certify that Oluwatobi A. Adeniji with matriculation number LCU/PG/001588 carried out this research work titled "Honey Induced Expression of Glutathione-Encoding Genes (*gsh*A and *gsh*B) in Clinical isolates of *Pseudomonas aeruginosa*" in the Department of Biological Science, Faculty of Applied Sciences, Lead City University, Ibadan, Oyo state, for the award of Master Degre (MSc) in Molecular Biology and Genomics and that this has not been previously submitted.

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Dr. F. C. Adesina **Date**

(Supervisor)

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Dr. F.C. Adesina **Date**

(Head of the Department)

Dedication

This research work is dedicated to the Almighty God for making its completion possible.

Acknowledgement

My sincere gratitude goes to the staff of the department of Biological Sciences Lead City University, Ibadan starting with my supervisor who is also the Head of department, Associate Professor F. C. Adesina, who despite her very busy schedules took out time to read through and correct my work. I am grateful for her support and encouragement all through the period of this work. I appreciate all my lecturers in the department for their patience and love while impacting me with knowledge during my course of study.

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Lastly, I want to state that even though the above-mentioned institutions and persons have assisted in the process of this research work, I alone stand responsible for the errors, if any, found in the work.

Abstract

Pseudomonas aeruginosa, a commonly isolated pathogenic and multidrug resistant bacteria contains *gshA* and *gshB* genes also present in most Gram-negative bacteria. The genes encode enzymes involved in glutathione biosynthesis important in biofilm formation, bacteria virulence and stress protection. The expressions of these genes have been shown to increase in the presence of oxidative stress due to H_2O_2 as well as other hydroperoxides. Honey has been found to have antibacterial properties against a wide range of bacteria species and H_2O_2 is the major contributor to the antimicrobial activity of most honey types especially at 30%-50% dilution. This experimental study isolated and assessed the antibiotics and honey susceptibility of some *Pseudomonas aeruginosa* strains from LTH Ogbomoso Nigeria. Investigation into their 16SrRNA for confirmation and assessment of the expression of the *gsh*A and *gsh*B genes in the presence of varying percentages (100%, 50% and 25%) of the sweet and bitter Nigerian Honey was done using Conventional and Real-time PCR. The presence and expression of these genes were detected in three out of the eight selected isolates of *Pseudomonas aeruginosa* in the controls(untreated), 50% and 25% honey dilutions and they showed down regulation of an average of 8.8 ±0.28 (25%) and 9.0 ±0.08 (50%) and 8.3±1.2(25%) and 7.0±0.5 (50%) fold decrease for *gsh*A and *gsh*B genes respectively in sweet honey, except in the 100% where they were not expressed. This study has been able to establish the presence of the glutathione producing genes *gsh*A and *gsh*B in clinical isolates of *Pseudomonas* aeruginosa. The expression of the genes mean that H_2O_2 present in honey is liable to deactivation by the production of glutathione. Glutathione genes should be considered as essential for the successful treatment of *Pseudomonas aeruginosa* infections signifying the importance of these genes in relation to honey therapy when used as an antibacterial agent.

Keywords: *Pseudomonas aeruginosa*; Gene Expression; *gsh*A and *gsh*B; Honey-resistance.

Word Count - 300

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Chapter Two: Literature Review

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List of Acronyms

CLSI- Clinical and Laboratory Standard Institute

$C_{T.}$ Cycle Threshold

EDTA - Ethylenediaminetetraacetic acid

PCR- Polymerase Chain Reaction

NAFDAC – National Agency for Food and Drug Administration and Control

TE – Tris-Cl EDTA

RT-PCR- Real-time PCR

qPCR- Quantitative Polymerase Chain Reaction

WHO- World Health Organization