IMMUNOMODULATORY POTENTIAL OF PROBIOTIC LACTIC ACID BACTERIA ISOLATED FROM THE SAP OF *Elaeis guineensis* (PALM WINE)

BY

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DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY

FACULTY OF PHARMACEUTICAL SCIENCES AGULU, NNAMDI AZIKIWE UNIVERSITY AWKA

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BEING A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY, FACULTY OF PHARMACEUTICAL SCIENCES AGULU, NNAMDI AZIKIWE UNIVERSITY, AWKA IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY

SUPERVISORS: PROF. C. O. ESIMONE AND DR. C. S. NWORU

OCTOBER, 2018

CERTIFICATION

I, EZE, CHRISTOPHER OSITA, a doctoral student in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Agulu, Nnamdi Azikiwe University, Awka with registration number 2011667003P do hereby certify that the work embodied in this project is original and has not been submitted in part or full for any other degree or diploma in this or any other University or college.

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EZE, CHRISTOPHER OSITA **DATE**

APPROVAL PAGE

This is to certify that this thesis has been approved by the Department of Pharmaceutical Microbiology and Biotechnology Postgraduate Board for the award of Doctor of Philosophy (Ph.D). This work is original; it has not been submitted in any form for the award of any degree, diploma or certificate in any University

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DEDICATION

I dedicate this work to our Lord Jesus Christ whose divine mercy saw to the completion of this work and to my mum for her continual encouragement in the field of academics.

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TABLE OF CONTENTS

ABSTRACT

The aim of this study was to investigate the immunomodulatory potentials of probiotic bacteria isolated from palm wine. The objectives were: to isolate and characterize the probiotic bacteria in palm wine; to determine the effect of the isolates on innate immunity; to determine the effect of the isolates on antibody secretion; and to determine the effect of the isolate on cell-mediated immunity.

The probiotic bacteria were isolated by streak plate method, characterized phenotypically using Gram staining, spore staining, catalase , motility and sugar fermentation tests, and genotypically using DNA extraction, PCR, gene sequencing and online blast of the sequenced gene with the help of established molecular biological tools. The identified probiotic bacteria were subjected to immunological studies for their effects on *in vivo* leukocyte mobilization rate (innate immunity), delayed-type hypersensitivity response (cell - mediated immunity) and antibody (IgG, IgG1 and IgG2a) secretion (humoral immunity) using sheep red blood cell as antigen. Their effects were considered at 4 and 9 days post-secondary antigenic challenge.

The following probiotic bacteria were identified:*Leuconostocmesenteroides, Leuconostocholzapfelii, Leuconostoccitreum, Leuconostockimchii, Leuconostoclactis, Leuconostoc fallax, Fructobacillusdurionis, Lactobacillus brevis, Lactobacillus paracasei subsp. tolerans, Lactobacillus paracasei and Lactobacillusyonginensis*. The isolates produced a percentage increase of 213, 204, 203, 188.6, 152.5,126.7, 115, 96.5, 91, 34.3 and 32.8% on the amount of leukocytes mobilized for *Lb. paracasei,Lb. yonginensis, Lc.holzapfelii, Lb.paracasei subsp. Tolerans, Lb. brevis, Lc. mesenteroides, Lc. kimchii, Lc. citreum, F. durionis, Lc. Fallax*and *Lc. Lactis*respectively while the positive control(linex capsule a brand of Lyophilized *Lactobacillus* spp) produced 83.8% increase when compared with the negative control. The isolates significantly affected positively, delayed type hypersensitivity response by 550, 800, 950, 600, 750, 600, 1150, 300, 600, 600, 500 and 650 % for *Lc. mesenteroides, Lc. holzapfelii, Lc. citreum, Lc. kimchii, Lc. lactis* and *Lc. fallax, F. durionis, Lb. brevis, Lb. paracasei subsp. Tolerans, Lb. paracasei, Lb. yonginensis*and positive control respectivelywhen compared with the negative control.The result of the ELISA showed that there was an insignificant increase in the antibody titre for the different LAB species 4 days post- secondary antigenic challenge for IgG. However at day 9 post-secondary antigenic challenge for IgG, there was marked increase of 137.8, 125.9, 122.9, 120.7, 118.3, 110.3, 106.4, 100.2, 67.9, 444.6 and 41.9% for *Ln. fallax, Ln. holzapfeli, Lb.paracasei sub tolerans, F.durionis, Lb.yonginensis, Ln. kimchi, Lb. paracasei, Lb. brevis, Ln. lactis, Ln. citreum and Ln.mesenteriodes* respectively when compared with the negative. However the effects of the isolates on IgG1 and IgG2a secretions were marginal and in some cases suppressive both at 4 and 9 days post-secondary antigenic challenge.

The results showed that the various species of probiotic bacteria isolated from palm wine possess immunomodulatory potentials as they were able to affect positively the different components of the immune system. Fresh palm wine can be an important source of probiotics.