ANTIBACTERIAL AND IMMUNOMODULATORY EFFECTS OF LEAF AND ROOT-BARK EXTRACTS FROM VERNOA HILDEBRANDTII, ACACIA STUHLMANNII (TAUB) AND MORINGA OLEIFERA

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any
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DEDICATION

This thesis is dedicated to my wife Fatma and my son Karima for their love, understanding and support during my study. Without their exemplary attitude towards life and intellectual spirit, I would not have been able to maintain the level of enthusiasm and motivation necessary to complete this academic journey.

I am greatly indebted to all my friends who in various ways, through their insightful comments, continuous support, patience, motivation, enthusiasm and encouragement led to the success of my research work.

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ABBREVIATIONS

DMSO: Dimethylsulfoxide

MTT: 3-(4, 5-Dimethylthiazol 2-yl)-2, 5-diphenyltetrazolium bromide

MIC: Minimum Inhibition concentration

WHO: World Health Organization

ATCC: American Type Culture Collection

MHA- Muller Hinton Agar

CLSI: Clinical and Laboratory Standard Institute

IZD: Inhibition Zone Diameter

PBMCs: Peripheral Blood Mononuclear Cells

PBS Phosphate buffered Saline

WBC: White Blood Cells

FBS: Fetal Bovine Serum

OD: Optical Density

ELISA: Enzyme Linked Immunosorbent Assay

PBS: Phosphate Buffered Solution

ABSTRACT

Over the years, the potential of plants as a source of new antimicrobial substances has been determined but is still under-explored. In Africa and most developing countries, traditional medicine still forms the backbone of rural medicinal practice. In addition, the emergence of new diseases and drug resistant microorganisms has highlighted an essential role of medicinal plants as a source for new healing agents in development of therapeutic drugs. This study aimed at determining the antibacterial immunomodulatory activity of Vernoa hildebrandtii, Acacia stuhlmannii (Taub) and Moringa oleifera leaf and root-bark extracts. Crude extracts were obtained from dried powder by single solvent maceration with ethanol and water. Bioassay-guided procedures were used to evaluate the bioactivity of the extracts against Escherichia coli and Staphylococcus aureus. Antibacterial activity was determined by agar diffusion method. Elisa was used to assay for Interleukin -10 and Interferon gamma production. In addition, (3-(4, 5-Dimethylthiazol 2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to determine cytotoxicity. Statistical analyses were performed using one way ANOVA followed by student t-test. Results were considered statistically significant at P < 0.05. M. oleifera leaf water extract was the most potent fraction with the highest zone of inhibition of 32 ± 2 mm followed by A. stuhlmannii (Taub) root-bark water extracts with a zone of inhibition of 31 ± 2 mm. A. stuhlmannii (Taub) leaf extracts did not show any antimicrobial activities against all the tested microorganisms. The plant extracts exhibited a dose dependent antibacterial effect on the E. coli and S. aureus bacteria. Human PBMCs treated with leaf and root-bark extracts showed secretion of IFN-y with M. oleifera root-bark alcohol extracts showing highest secretion as compared to the controls while down regulating IL-10. The extracts significantly increased cell proliferation in a dose-dependent manner as demonstrated by the increase in cell viability probably due to low cytotoxicity levels. V. hildebrandtii leaf water extract had the highest cell proliferation with a mean cytotoxic concentration (CC₅₀) value of 42.22 µg/ml, A. stuhlmannii (Taub) leaf alcohol and Moringa oleifera leaf water extracts both recording a CC₅₀ value of 37.77 µg/ml. The toxicity results showed that all the extracts except Moringa oleifera root-bark water were within the accepted cytotoxicity levels (CC_{50} 20 µg/ml toxic > non-toxic). Taken together, these results confirm that M. oleifera have antibacterial properties against E. coli and S. aureus bacteria. Further, they demonstrate the potential of V. hildebrandtii and Acacia stuhlmannii (Taub) as new sources of bioactive agents in the management of E. coli and S. aureus pathogens. The findings further demonstrate for the first time, to the best of our knowledge, that V. hildebrandtii has a very high selective potential for antibacterial activity. Further, there was a considerable degree of immunomodulation as demonstrated by up-regulation of IFN-y and down-regulation of IL-10 as the plant extract concentration increased, meaning cell mediated immune response is stimulated.