Antilisterial bioactivity and /or biofilm-formation by compounds from *Plectranthus ecklonii* Benth. and *Acacia karroo* Hayne

By

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DECLARATION

I, Monde Alfred Nyila declare that the thesis, which I hereby submit for the degree Doctor Philosophiae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE……

DATE……..

List of Abbreviations

BHI	Brain heart infusion
CFU	Colony forming units
CSLM	Confocal scanning laser microscopy
DMSO	Dimethyl sulphoxide
GI	Growth index
IC_{50}	Fifty percent inhibitory concentration
INH	Isoniazid
INT	p-iodonitrotetrazolium violet
MBC	Minimum bactericidal concentration
MDR	Multidrug-resistant
MEM	Minimum Essential Medium
MIC	Minimum inhibitory concentration
NMR	Nuclear magnetic resonance
SI	Selectivity index
TB	Tuberculosis
TLC	Thin layer chromatography
TSA	Tryptone soya agar
TSB	Tryptone soya broth
XTT	2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2-H-tetrazolium hydroxide

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Abstract

Thirteen South African medicinal plants which are used traditionally to treat symptoms associated with *Listeria monocytogenes* infections, were screened for activity against the pathogen. Different plant parts were extracted separately with ethyl acetate or chloroform. All the extracts were first screened against the bacteria using the disc diffusion method. Zones of inhibition observed in the presence of the chloroform extracts of *Eucomis autumnalis*, ethyl acetate extracts of *Acacia karroo* and *Plectranthus ecklonii* (50 mg/ml) were 12 mm, 14 mm and 15 mm respectively. Active extracts were further tested against the bacteria for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the microtitre dilution method. Ethyl acetate extracts of *A. karroo* exhibited MIC of 3.1 mg/ml and MBC of 6.25 mg/ml. Ethyl acetate extracts of *P. ecklonii* showed MIC of 0.5 mg/ml and MBC of 1.0 mg/ml. Five samples namely *A. karroo* (ethyl acetate extract), *P ecklonii* (ethyl acetate extract), *Senecio inonartus* (ethyl acetate extract), *S. inonartus* (chloroform extract) and *Aloe arborescens* (ethyl acetate extract) showed good MIC against *L. monocytogenes* and a MBC range from 1.0 to 12.5 mg/ml. The two plants, *A. karroo* and *P. ecklonii* were further selected for the isolation of the active compound(s).

Column chromatographic purification of ethyl acetate extracts of the leaves of *A. karroo* led to the isolation of three known pure compounds namely β-sitosterol, epigallocatechin and epicatechin. The MICs of the β-sitosterol and epigallocatechin that were isolated from *A. karrroo* were found to be 31.25 µg/ml and 62.5 µg/ml respectively against *L. monocytogenes.* The confocal scanning laser microscopy (CSLM) showed that the biomass of the listerial biofilms were reduced when the isolated compounds were added and slightly

reduced when the crude extract was added. The aggregation of cells which were exposed to β-sitosterol and epigallocatechin was reduced from 25 µm as observed in untreated cells to < 10 µm in length. Therefore as one of the local South African plants identified in the present study, the pure compounds isolated from *A. karroo,* could be used as a potential natural alternative for eliminating *L. monocytogenes* biofilms from food processing surfaces. This could help in combating the problem of food contamination and food poisoning caused by the pathogen. It could also help in preparing antibiofilm agents that are cost effective and easily accessible to the public. *A. karroo* should be further be explored in this regard. The present study reports for the first time the isolation of the three compounds, β-sitosterol, and epicatechin and epigallocatechin from *A. karroo*.

Bioassay-guided fractionation of the *P. ecklonii* ethyl acetate extract led to the isolation of two known compounds, parvifloron D and parvifloron F. Parvifloron D and F exhibited a minimum inhibitory concentration (MIC) of 15.6 and 31.25 µg/ml respectively against *L. monocytogenes.* The MICs of parvifloron D and F against a drug-sensitive strain of *Mycobacterium tuberculosis* were found to be 190 and 95 µg/ml respectively. The ethyl acetate extract of *P. ecklonii* and its isolated compounds were tested for their activity on tyrosinase inhibition. The concentration of plant extract at which half the tyrosinase activity was inhibited (IC_{50}) was found to be 61.73 ± 2.69 µg/ml. The antibacterial activity of the extract of *P. ecklonii* and its isolated compounds correlates with the traditional use of the plant for various ailments such as stomach-aches, diarrhoea and skin diseases. This is the first report on the bioactivity of an extract of *P. ecklonii* and its two compounds.

The antibacterial activity of the extracts of *A. karroo*, *P. ecklonii* and their isolated compounds correlates with the traditional use of these plants for symptoms associated with listeriosis.