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Activity of South African medicinal plants against *Listeria monocytogenes* biofilms, and isolation of active compounds from *Acacia karroo*

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Abstract

In South Africa, the antimicrobial activity of many indigenous plants has been investigated. In general, studies have focused on planktonic bacteria, with less attention given to bacterial biofilms. Many organisms, however, including the opportunistic pathogen *Listeria monocytogenes* occur more frequently as biofilms. The aim of this study was to identify and select plants that exhibit the best antilisterial activity, isolate the bioactive compounds and determine their effect on the architecture of listerial biofilms. The ethyl acetate and chloroform extracts of thirteen plants were investigated for antilisterial activity. The ethyl acetate extract of *Acacia karroo* and *Plectranthus ecklonii* showed the best antilisterial activity, exhibiting a minimum inhibitory concentration (MIC) of 3.1 mg/ml and 0.5 mg/ml, respectively. These were further selected for the identification of bioactive compounds. Column chromatographic purification of the ethyl acetate extracts of the leaves of *A. karroo* led to the isolation of three known pure compounds, namely epicatechin (**1**), β-sitosterol (**2**) and epigallocatechin (**3**). Confocal scanning laser microscopy (CSLM) showed that the biomass of the listerial biofilm was reduced when the isolated compounds were added. The aggregation of cells that were exposed to β-sitosterol and epigallocatechin was reduced from 25 μm as observed in untreated cells to <10 μm in length.

Keywords: Antilisterial activity; Biofilms; Confocal Scanning Laser Microscopy (CSLM); *Listeria monocytogenes*; Medicinal plants; South Africa

1. Introduction

Listeria monocytogenes is the causative agent of listeriosis, a severe human infection characterised by gastro-enteritis, meningitis, encephalitis, septicaemia and spontaneous abortions (De Souza et al., 2008; Goldenberg and Thompson, 2003; Prescott et al., 2005). Individuals most prone to the disease are pregnant women, newborns, the elderly and immuno-compromised patients, including those with HIV (Békondi et al., 2006; DiMaio, 2000; Prescott et al., 2005). In pregnancy, infection with this organism may cause complications and lead to stillbirths or spontaneous abortions (DiMaio, 2000; Goldenberg and Thompson, 2003). Local plant species such as *Artemisia afra*, *Acacia karroo*, *Ziziphus mucronata* and *Eucomis autumnalis*, have been used extensively for the treatment of listeriosis related symptoms (Van Wyk and Gericke, 2000; Van Wyk et al., 2009) while globally, *Camellia sinensis* (Si et al., 2006), *Ruta graveolens* (Alzoreky and Nakahara, 2003), *Mutisia acuminata* var. *acuminata* (Catalano et al., 1998) have also been found to have antilisterial activity.

Current research on these medicinal plants has placed greater emphasis on their antimicrobial activity against free floating cells (planktonic) with less focus on biofilms which are associated

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with severe infections (Quave et al., 2008). Bacterial biofilms are more resistant to the action of antimicrobial and disinfectant agents (García-Almendárez et al., 2007). This resistance (Nichterlein et al., 1998; White et al., 2002) has resulted in the need for multi-drug treatment associated with higher incidence of side effects and increased toxicity (Cone et al., 2003; Gleckman and Borrego, 1997). The aim of this study was to identify and select the plants that exhibited the best antilisterial activity, isolate the bioactive compounds and determine their effect on the architecture of listerial biofilms.

2. Materials and methods

2.1. Plant material

Thirteen South African medicinal plants were collected from Gauteng and the Free State. Different parts of the plants (i.e. leaves, stem, bark, roots) were collected (Table 1). The plant collection was based on information received from experienced traditional healers, elderly indigenous people (who are experts in traditional medicine) and from the literature (Lukhoba et al., 2006; Van Wyk and Gericke 2000; Van Wyk et al., 2009). Plants were taken to HGWJ Schweickerdt Herbarium at the University of Pretoria for identification, and herbarium specimens were preserved.

2.2. Preparation of plant crude extracts

Ethyl acetate and chloroform extracts of each plant sample were prepared. To obtain these extracts, 100 g of fresh plant material was homogenised and extracted with 1000 ml of ethyl acetate or chloroform. The extract was filtered and concentrated. The residue was later dissolved in 10% DMSO to a final stock concentration of 50 mg/ml.

2.3. Bacterial strain and preparation of inoculum

A pathogenic strain of *Listeria monocytogenes* (LMG 21263) used in this study was obtained from the Department of Pharmaceutical Sciences at Tshwane University of Technology. It was activated by transferring a loopful from the Brain Heart Infusion (Merck) slants into Tryptone Soya Broth (Merck), followed by incubation at 37 °C for 24 h. The bacterial counts of the standardised culture were confirmed by plating out on Tryptone Soya Agar (TSA, Merck) plates and incubating at 37 °C for 24 h. Stock cultures were maintained at −70 °C (Alzoreky and Nakahara, 2003).

2.4. Disc diffusion antimicrobial assay

The disc diffusion method, as described by Alzoreky and Nakahara (2003), was used for testing the susceptibility of

Table 1
Voucher specimen numbers of selected plants and their ethnobotanical uses.

Plant species	Family	Ethnobotanical uses	Key references	Voucher specimens number
Acacia karroo Hayne	*Fabaceae*	Used medicinally to treat diarrhoea, colds, dysentery, conjunctivitis and haemorrhage.	Van Wyk and Gericke 2000; Van Wyk et al., 2009	MN 15
Aloe arborescens Mill.var. *natalensis* Berger	*Asphodelaceae*	Used to treat wound and burns. Also has been reported to be used during pregnancy to ease labour.	Grace et al., 2008; Van Wyk et al., 2009	MN 5
Artemisia afra Jacq.	*Asteraceae*	Used to treat coughs, fever, colds, influenza and blocked nasal passages.	Van Wyk and Gericke 2000; Van Wyk et al., 2009	MN 7
Clivia miniata Reg.	*Amaryllidaceae*	Used to treat fever and to relieve pain while the whole plant is used in childbirth.	Louw et al., 2002; Van Wyk et al., 2009	MN 3
Datura stramonium L.	*Solanaceae*	Used to relieve asthma, rheumatism, gout, boils, abscesses, sore throat, tonsillitis and respiratory difficulties.	Van Wyk et al., 2009	MN 8
Drimia altissima (L.f.) Ker Gawl	*Hyacinthaceae*	Used as expectorants (promoting secretion, liquefaction or expulsion of the sputum from the respiratory passages, emetics, diuretic and as a heart tonic, to treat fever, bladder and uterus disease.	Louw et al., 2002; Van Wyk et al., 2009	MN 14
Eucomis autumnalis (Mill.) Chitt.	*Hyacinthaceae*	Used for fever, stomach ache, colic, urinary diseases, diarrhoea, syphilis, easing of child birth, chest complaints, coughing and tuberculosis.	Louw et al., 2002; Van Wyk, 2008; McGaw et al., 2008; Van Wyk et al., 2009	MN 11
Gomphocarpus fruticosus (L) W.T. Aiton	*Asclepiadaceae*	Used medicinally to treat headaches, stomach pain, tuberculosis and as an emetic.	Van Wyk et al., 2009	MN 1
Heteromorpha arborescens (Spreng) Cham. &. Schltdl.	*Apiaceae*	Used to treat headaches, fever, asthma, coughs, dysentery, infertility, abdominal pains and colic.	Van Wyk et al., 2009; Lundgaard et al., 2008	MN 4
Plectranthus ecklonii Benth.	*Lamiaceae*	Used to treat stomach aches, nausea, vomiting and meningitis.	Lukhoba et al., 2006	PRU 96396
Senecio inonartus DC	*Asteraceae*	Used to treat various illnesses such as chest pains, headaches, swellings, burns and sores.	McGaw et al., 2008; Van Wyk et al., 2009	MN 9
Tulbaghia violaceae Harv.	*Alliaceae*	Used to treat fever, colds, asthma and tuberculosis.	Van Wyk, 2008; Van Wyk et al., 2009	MN 2
Ziziphus mucronata Wild.	*Rhannaceae*	Used to treat coughs, chest problems, and diarrhoea.	Van Wyk and Gericke, 2000; Van Wyk et al., 2009	MN 10

L. monocytogenes to the plant extracts. Two hundred microlitres of prepared culture (10^6 CFU/ml) was spread on the surface of Mueller–Hinton agar plates. Sterile filter paper discs (10 mm in diameter) were impregnated with 2.5 mg of the extracts. Erythromycin (7.5 μg) was used as a positive drug control. Plates were then kept at ambient temperature for 30 min to allow diffusion of the extracts prior to incubation at 37 °C for 24 h.

2.5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

Ethyl acetate and chloroform extracts of *Acacia karroo*, *Eucomis autumnalis*, *Drimia altissima, Aloe arborescens*, *Plectranthus ecklonii* and *Senecio inonartus*, which showed antilisterial activity in the initial screening using the disc diffusion method, were further evaluated to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using 96-well microtitre plates. The microdilution method as described by Eloff (1998) was used. Briefly, the extracts were first dissolved in 10% DMSO and then added to Tryptone Soya Broth (TSB) to obtain a final concentration of 25 mg/ml in the first well. A serial double dilution was performed to obtain a concentration range of 25–0.01 mg/ml for the extracts. The antibiotic erythromycin (Merck) at concentrations ranging from 150–0.29 μg/ml served as a positive drug control. A hundred microlitres of bacterial inoculum (10^6 CFU/ml) of *L. monocytogenes* was added to the wells and thereafter the plates were incubated at 37 °C for 24 h. After 24 h incubation microbial growth was tested by adding 40 μl of (0.2 mg/ml) *p*-iodonitrotetrazoilium violet (INT) (Sigma-Aldrich, South Africa) to the microtitre plate wells and re-incubated at 37 °C for 1 h. A change in colour to pink indicated that the cells were still viable. The MIC was defined as the lowest concentration of the extract that caused no colour change. To determine the minimum bactericidal concentration (MBC) against *L. monocytogenes*, 50 μl of the sample (from the wells which did not show bacterial growth during MIC determination) were transferred into 150 μl of freshly prepared TSB and spread onto Tryptone Soya Agar (TSA) plates to detect growth. The plates were then re-incubated for another 24 h and the MBC (lowest dilution of extracts with no growth after 24 h incubation at 37 °C) was determined according to Reimer et al. (1981).

A. karroo and P. ecklonii warranted further investigation for the isolation and identification of active compounds. The isolation of compounds from *P. ecklonii* and their antilisterial activity have been recently reported (Nyila et al., 2009). In the present study, the isolation of compounds from the leaves of *A. karroo* and their antilisterial activity are reported.

2.6. Isolation and identification of active compounds

Leaves of *A. karroo* (1.2 kg) were extracted with ethyl acetate (2×2 l) for 48 h at room temperature. The ethyl acetate extract was filtered and concentrated under vacuum. The total extract (72.7 g) was subjected to silica gel column chromatography (CC) using 1000 ml of hexane/ethyl acetate mixtures of increasing polarity (0%, 20%, 40%, 70%, 100% ethyl acetate; v/v) followed by 100% methanol (MeOH). Similar fractions were combined according to the TLC profile into 12 main fractions.

A direct bioassay on the TLC plate was done by applying 20 μl of the fraction (50 mg/ml) to the silica gel 60 F_{254} plate (Simonovska and Vovk, 2000). The plates were developed using ethyl acetate: ethanol (9:1, v/v) eluent followed by careful drying. A 24 h *L. monocytogenes* culture in TSB was centrifuged at 1000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved in fresh nutrient broth. The bacterial suspension was applied as a fine spray to the developed TLC plates according to Meyer and Dilika (1996) and incubated at 37 °C for 24 h. The plates were then sprayed with 0.2 mg/ml INT. Bacteria on the plate changed the INT into pink coloured formazan and inhibition of bacteria resulted in no colour change (Hamburger and Cordell, 1987). Fractions IV, V and XII showed good zones of bacterial inhibition in the direct bioautographic assay and were subjected to further column chromatographic purification. Fraction IV was subjected to column chromatographic using a Sephadex column to yield compound **1** (yield 24 mg). Fraction V was subjected to silica gel chromatography using hexane: ethyl acetate (9:1; v/v) as eluent which resulted in the pure compound **2** (yield 600 mg). Fraction XII, purified under the same conditions, resulted in pure compound **3** (yield 128.4 mg). ^{1}H NMR and ^{13}C NMR spectra were recorded using a Brucker ARX 300 or a Brucker Avance DRX 500 MHZ using $CDCl_3$, and DMSO-d_6 as solvents.

2.7. Cytotoxicity assay

Microtitre plates with Vero cells were used for testing the selected extracts for cytotoxicity following the method of Zheng et al. (2001). Cytotoxicity was measured by using a XTT-based colorimetric assay Proliferation kit II (Roche Diagnostics GMbH). The Vero cells (100 μl) were seeded out at 10^5 cells/ml onto a microtitre plate and incubated for 24 h to allow the attachment and settling of the cells at the bottom of the plate. A serial dilution was made from the selected extracts and purified compounds of *A. karroo* (final concentration 200–0.2 μg/ml) were added to the microtitre plate and incubated for 48 h. The XTT reagent (50 μl of 0.3 mg/ml) was added and the cells were again incubated for 1–2 h to allow for colour development. The drug control Zearalenone (Boehringer-Ingelheim, Germany), at a final concentration of 1.25 μg/ml, was included as a positive control. After incubation the absorbance of the colour complex was spectrophotometrically quantified using an ELISA plate reader, which measured the optical density at 490 nm with a reference wavelength of 690 nm. The GraphPad Prism 4 statistical programme was used to analyse the fifty percent cytotoxic concentration (IC_{50}) values.

2.8. Confocal Scanning Laser Microscopy (CSLM)

A modified method (Kives et al., 2005) was used for investigating the effect of the antilisterial extracts of *A. karroo* and its purified compounds on listerial biofilm formation. Briefly, a standardised overnight culture was allowed to develop a biofilm on glass slides that had been previously coated with 100 μl TSB to provide nutrients for the adhering bacteria (Chae and Schraft, 2000). These were then placed in sterile Petri dishes and incubated at 37 °C for a

further 24 h. After incubation these glass slides were either left untreated (negative control), treated with erythromycin (positive control, 0.15 mg/ml), ethyl acetate extract of *A. karroo* (1.0 mg/ml) or pure compounds (0.5 mg/ml). After 24 h incubation, the slides were removed from the incubator and the samples were prepared for viewing with the Zeiss LSM 510 META (Carl Zeiss, Jena, Germany). Samples were prepared as follows: 1 µl of propidium iodide was combined with 19 µl of sterile double distilled water and poured over the biofilm containing slide. The use of propidium iodide facilitated viewing of the listerial biofilm under the CSLM. The presence or reduction of the biofilm on the glass slide indicated whether the samples were active against the *L. monocytogenes* cells.

3. Results and discussion

In this study, only five of the 13 plant species (Table 1) tested were active against *L. monocytogenes* in the disc diffusion bioassay. The most active plant extracts against *L. monocytogenes* were from *A. karroo* (14 mm inhibition) and *P. ecklonii* (15 mm inhibition) (Table 2) while *S. inonartus* and *A. arborescens* also had limited inhibition at a concentration of 2.5 mg per disc. The crude extracts that showed inhibitory activity in the disc diffusion bioassay were selected for further tests against *L. monocytogenes*. The five plants, namely *A. karroo* (ethyl acetate extract), *P. ecklonii* (ethyl acetate extract), *Senecio inonartus* (ethyl acetate extract), *Aloe arborescens* (ethyl acetate extract) and *E. autumnalis* (chloroform extract) exhibited good minimum bactericidal activity against *L. monocytogenes,* with the MBC ranging between 0.5 mg/ml and 12.5 mg/ml in the micro-dilution assay. The MIC value for the *A. karroo* crude extract at 3.1 mg/ml showed good antilisterial activity (Table 3). This is similar to results obtained by Alzoreky and Nakahara (2003) on Asian plant extracts. Alzoreky and Nakahara (2003) reported MIC values ranging from 1.32 to 2.64 mg/ml of buffered methanolic extract of *Artemisia absithium* and eighteen other plants. The alcohol extract of *Rhus coriaria* showed an MIC of 2.5 mg/ml (Nasar-Abbas and Halkman, 2004).

The three fractions of *A. karroo* which were subjected to chromatography resulted in the identification of (**1**) epicatechin (flavonoid)**,** (**2**) β-sitosterol (phytosterol) and (**3**) epigallocatechin (flavonoid) (Fig. 1). Compound **1,** which gave a reddish brown spot using vanillin/H_2SO_4 reagent on TLC was isolated from fraction XII. The compound was identified using ^{1}H-NMR data. The ^{1}H-NMR data of **1** exhibited signals identical with that of epicatechin which exhibited signals of 6 protons, at δ 4.85 assigned to H-2, a proton signal δ 4.18 ppm, to H-3, and the two protons signals at 2.74 and 2.80 ppm were assigned to protons 4α and 4β, respectively. The signal at δ 6.01 was assigned to H-6 and the signal at 5.90 to H-8 (Okushio et al., 1998). Aromatic signals at δ_H 7.02, 6.75, and δ_H 6.80 correspond with that of a B-ring. The basic structure was derived as a 3,3′, 4′,5,7-pentahydroxyflavan, and the broad proton singlet at δ_H 4.82 indicated the compound as epicatechin (Sun et al., 2006).

From Fraction V one pure compound (**2**) was obtained. This compound was identified as β-sitosterol when spectra were compared to published data (Prozesky, 2004). Compound **2**

Table 2
Antilisterial activity of the plant extracts (2.5 mg/disc) against *Listeria monocytogenes* (LMG 21263) as determined by the disc diffusion method.

Plant species	Plant part used	Zone of inhibition (mm) [a]	
		Ethyl acetate	Chloroform
Acacia karroo Hayne	Leaves	14	12
Aloe arborescens Mill.var. *natalensis* Berger	Leaves	9	8
Artemisia afra Jacq.	Leaves	nz [b]	nz
Clivia miniata Reg.	Whole plant	nz	nz
Datura stramonium L.	Leaves	nz	nz
Drimia altissima (L.f.) Ker Gawl	Roots	nz	nz
Eucomis autumnalis (Mill.) Chitt.	Bulb	nz	nz
Gomphocarpus fruticosus (L) W.T. Aiton	Leaves	nz	nz
Heteromorpha arborescens (Spreng) Cham. &. Schltdl.	Stem Leaves	nz	nz
Plectranthus ecklonii Benth.	Leaves	15	12
Senecio inonartus DC	Leaves	8	8
	Stem	8	nz
Tulbaghia violaceae Harv.	Leaves	nz	nz
Ziziphus mucronata Wild.	Leaves	nz	nz
Erythromycin (150 µg/ml)(drug control)		14	

[a] Zone of inhibition (mm): Zone of inhibition in millimetres.
[b] nz : no zone of inhibition observed (extract not active against *L. monocytogenes*).

Table 3
The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the crude extracts of *A. karoo*, *E. autumnalis*, *S. inonartus*, *A. arborescens* and *P. ecklonii* against *L. monocytogenes* and their IC_{50} values against Vero monkey cell lines.

Compounds/extracts	MIC (mg/ml)	MCB (mg/ml)	IC_{50} (µg/ml)± SD [a]
Epicatechin (**1**)	>0.5	>0.5	>200.0
β-sitosterol (**2**)	0.031	0.125	63.820±1.614
Epigallocatechin (**3**)	0.062	0.25	28.910±1.525
A. karroo			
Ethyl acetate	3.1	3.1	45.490±7.86
Chloroform	6.25	6.25	NT
P. ecklonii			
Ethyl acetate	0.5	1.0	30.125
Chloroform	6.25	6.25	NT
E. autumnalis			
Ethyl acetate	12.5	12.5	NT
Chloroform	12.5	12.5	NT
S. inornatus			
Ethyl acetate	12.5	12.5	108.400±0.995
Chloroform	12.5	12.5	99.940±4.191
D. altissima			
Ethyl acetate	12.5	12.5	NT
Chloroform	12.5	12.5	NT
A. arborescens			
Ethyl acetate	12.5	12.5	>400.0
Chloroform	12.5	12.5	
Erythromycin (antibacterial drug control)	1.7×10^{-3}	1.7×10^{-3}	14.380
Zearalenone (positive drug for cytotoxicity)	NA	NA	2.318±0.301

NT: not tested; NA: not applicable.
[a] IC_{50}: fifty percent cytotoxic values.

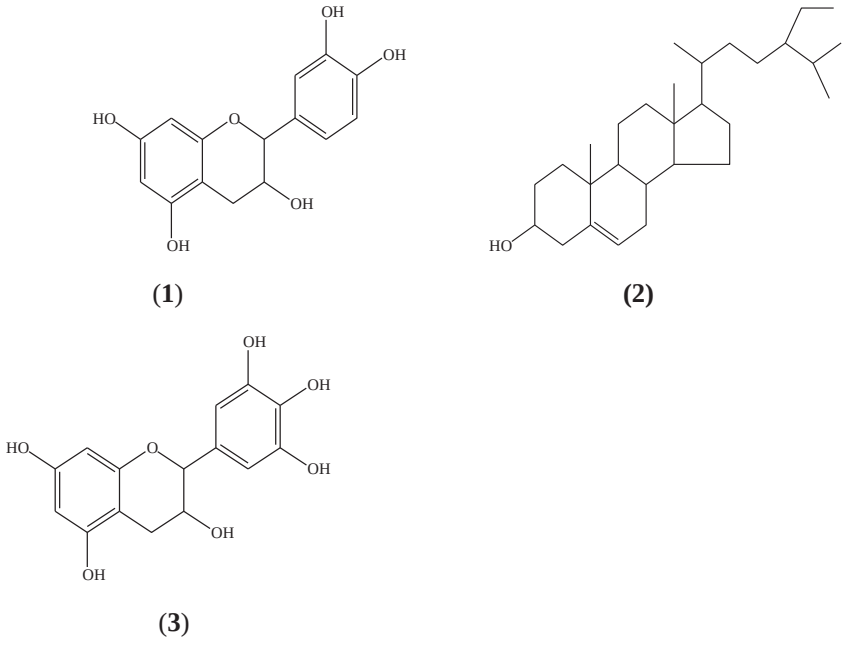

Fig. 1. Chemical structures of compounds isolated from the ethyl acetate extract of the leaves of *A. karroo*, (**1**) epicatechin; (**2**) β-sitosterol; (**3**) epigallocatechin.

has previously been isolated from *Raulinoa echinata* (Biavatti et al., 2001; Chattopadhyay et al., 2002; Misra et al., 2008) and *Acacia farnesiana* (Garcia et al., 2006). Fraction IV contained one pure compound (**3**) as determined by TLC and other spectroscopic data. The ^{1}H-NMR spectrum showed four aromatic protons at δ_H 5.68 (d, J=2.3), 5.87 (d, J=2.3), 6.38 (2H, d, J=0.6), two methyl protons at δ_H 4.86 (d, J=0.8 and 3.77 (s br), methyl protons at δ_H 2.45 (J=4.4) and 2.33 (J=3.2). The ^{13}C-NMR spectra indicated the presence of two methyl carbons attached to an oxygen function (δ_C 78.6, 65.6), a methyl carbon (δ_C 28.8 t), 12 aromatic carbons δ_C 156.4 (s), 156.2, 156.2, 155.3 (d), 145.7 (× 2C, d each), 132.5, 129.9, 106.0 (× 2C, s each), 99.0. The coupling constant between protons at δ_H 4.86 and 3.77 is 2.3=Hz which indicated ββ relative configuration. The above spectroscopic data indicated that compound **3** is epigallocatechin (brown powder). The present study reports for the first time the isolation of epicatechin (**1**), β-sitosterol (**2**) and epigallocatechin (**3**) from *A. karroo*.

The MIC value of β-sitosterol (**2**) in the present study was found to be 31.25 μg/ml while a bactericidal effect against *L. monocytogenes* was observed at a concentration of 125 μg/ml (Table 3). This compound has been shown, in previous studies (Chattopadhyay et al., 2002), to have antibacterial activity against various Gram-positive and Gram-negative bacteria. The phytosterol, β-sitosterol (**2**) showed more activity against *L. monocytogenes* compared to other pure compounds (5-amino-8-hydroxy-1,4-naphthoquinone) reported by Riffel et al. (2002) (MIC not determined as the compound was not active during disc diffusion). Adeniyi et al. (2000) reported MICs of diospyrin and isodiospyrin to be 0.05 mg/ml and 0.1 mg/ml respectively against *L. monocytogenes*. β-sitosterol (**2**) was found to be less toxic than epigallocatechin (**3**). The IC_{50} of β-sitosterol and epigallocatechin were found to be 63.82 and 28.91 μg/ml, respectively.

Epicatechin (**1**) and epigallocatechin (**3**) are known flavonoids that have been previously isolated from green tea (Gamberucci et al., 2006; Si et al., 2006) and from other plants (Chirinos et al., 2008; Sivakumaran et al., 2004). Epicatechin (**1**) and epigallocatechin (**3**) have been previously isolated from the leaves of *Lotus pedunculatus* (Foo et al., 1997). Yam et al. (1997) reported that *L. monocytogenes* was resistant to the antimicrobial activity of green tea at a concentration of 6 mg/ml. In contrast, in the present study, epigallocatechin (**3**) was found to be active against *L. monocytogenes* at a concentration of 0.062 mg/ml.

The relatively low MICs (Table 2) exhibited by epigallocatechin (**3**) warrants further investigation for use as a natural antilisterial medicinal product. It has been reported that compounds such as *N*-benzyl succinimide, with the lowest MICs are toxic in the green monkey Vero cell line (Zentz et al., 2004). This study showed that the triterpene, β-sitosterol showed the lowest MIC value (Table 3) and moderate cytotoxicity compared to the control drug.

The selectivity index (SI=IC_{50}/MIC) was found to be to 2.05 and 0.466 for compounds β-sitosterol (**2**) and epigallocatechin (**3**) respectively. The SI was not determined for Epicatechin (**1**). Compared to the other isolated compounds, beta-sitosterol (**2**) had the highest SI value and was found to be more selective than epigallocatechin (**3**). This is in accordance with literature (Kovala-Demertzi et al., 2009) where the compound with the highest activity was also found to be more selective (high SI value) than the compound with lower activity.

The aggregation of cells exposed to the pure compounds viewed with the CSLM showed a decrease compared to the untreated cells or the control (Fig. 2A). The untreated cells formed a biofilm while the cells treated with β-sitosterol (**2**) inhibited the formation of a listerial biofilm (Fig. 2B). Chi-Hua-Wu et al. (2008) reported on the antibacterial effects of American cranberry (*Vaccinium macrocarpon*) concentrate

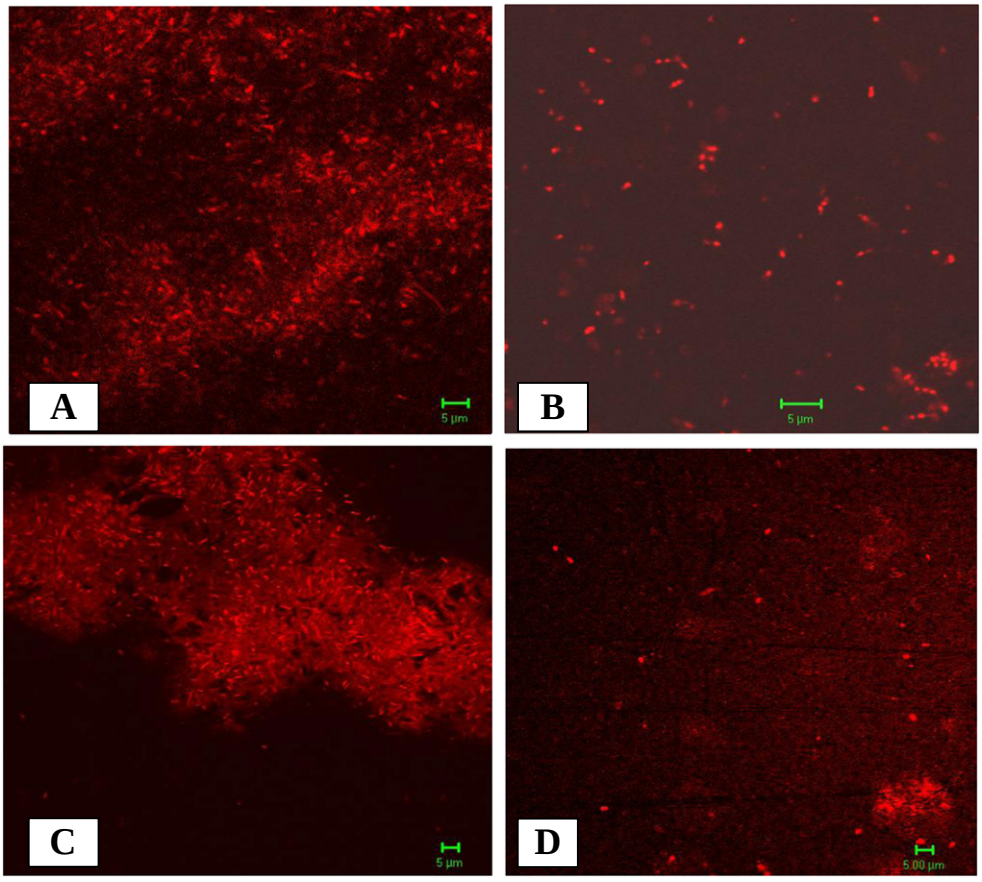

Fig. 2. CSLM images of *L. monocytogenes* (LMG21263) biofilm development on glass slides: (A) bacteria not exposed to plant extracts or compounds (negative control) showing a biofilm; (B) bacteria exposed to the compound, β-sitosterol (**2**) (500 μg/ml) showing a reduction in cell numbers and no development of a biofilm; (C) treatment with *A. karroo* crude extract showing biofilm (1.0 mg/ml) caused by cells as they adhere to each other; and (D) Treatment with epigallocatechin (**3**) (500 μg/ml) show no development of a biofilm.

on the foodborne pathogen, *L. monocytogenes*. In that study, the results of transmission electron microscopy showed that the bacterial cell wall was damaged (Chi-Hua-Wu et al., 2008). These results are in agreement with the previous report (Romanova et al., 2007) where the use of benzalkonium chloride, a quarternary ammonium compound reduced the formation of *L. monocytogenes* biofilms. Sandasi et al. (2008) reported that the use of selected individual essential oil components (EOC) enhanced the growth of a biofilm. In contrast, in this study the *A. karroo* crude extract (Fig. 2C) showed a slight reduction in the development of the listerial biofilm. Furthermore, both β-sitosterol (**2**) (Fig. 2B) and epigallocatechin (**3**) (Fig. 2D) had greater activity in the disruption of the *L. monocytogenes* biofilm. Epicatechin (**1**) did not reduce the biofilm. This is in contrast to other reports (Nostro et al., 2010; Simões et al., 2010) where it has been shown that most often the antimicrobial effect is due to the interaction of the individual compounds in the crude extract and not due to the individual compounds. Both β-sitosterol (**2**) and epigallocatechin (**3**) could potentially play a role as disinfectant agents against listerial biofilms and could be used for cleaning food processing surfaces and utensils. In the United States, the two compounds would meet the requirements of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (EPA, 2010) since they are natural products that are environmentally friendly (Chandramu et al., 2003; García-Marino et al., 2006; Row and Jin, 2006; Sajfrtová et al., 2010; Siripatrawan and Harte, 2010) and would be generally recognised as safe (GRAS) (EPA, 2010). Thus, the pure compounds isolated from *A. karroo,* could be useful as potential natural alternatives for eliminating *L. monocytogenes* biofilms from food processing surfaces. This could alleviate the problem of food contamination and poisoning caused by the pathogen. This plant could also aid in preparing anti-biofilm agents that are cost effective and easily accessible to the public.

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