RAPID BACTERICIDAL EFFECT OF CINNAMON BARK ESSENTIAL OIL AGAINST *PSEUDOMONAS AERUGINOSA*

E.R. Elcocks[a], P.T.N. Spencer-Phillips[a] and E.C. Adukwu[a]#

[a]Centre for Research in Biosciences, Faculty of Health and Applied Sciences, University of the West of England, Bristol, United Kingdom, BS16 1QY.

Running head: Cinnamon essential oil antimicrobial action

#Address correspondence to Dr E. C. Adukwu, Emmanuel.Adukwu@uwe.ac.uk.

KEYWORDS

Essential oils, plant extract, *Pseudomonas aeruginosa,* cinnamon, antimicrobial resistance, time-kill, multi-drug resistance, GC-MS.

ABSTRACT

AIMS

This study aimed to identify the most effective antimicrobial from a selection of essential oils and investigate its bactericidal properties against *Ps. aeruginosa*.

METHODS AND RESULTS

The disc diffusion assay and minimal inhibitory/bactericidal concentration tests were used to identify antimicrobial potential. Several oils exhibited antimicrobial effects at concentrations as low as 0.03% (v/v). Significantly, cinnamon (*Cinnamomum zeylanicum)* bark oil exhibited broad spectrum activity against Gram-negative and Gram-positive bacteria and showed bacteriostatic and bactericidal effects against *Ps. aeruginosa* PAO1 at 0.125% (v/v) and all other tested organisms, including known multidrug resistant species. Time-kill assays and metabolic activity tests showed cinnamon oil to exhibit rapid killing, with bactericidal activity observed in ≤6 min at ≥0.5% (v/v). Furthermore, scanning electron microscopy and a membrane permeability assay indicated damage to membrane integrity, loss of turgor and cell collapse.

CONCLUSION

Cinnamon bark essential oil is a broad-spectrum antimicrobial capable of rapid killing at low concentrations.

SIGNIFICANCE AND IMPACT OF STUDY

This study provides a sound basis for further investigation of the potential of cinnamon bark essential oil as an alternative to conventional antimicrobial products due to its fast acting bactericidal properties at low concentrations.

INTRODUCTION

Approx. 100,000 tonnes of antibiotics are manufactured globally per year (Nikaido 2009). Not only have bacteria developed antimicrobial resistance (AMR), many strains have become resistant to multiple antibiotics and chemotherapeutic agents, termed multi-drug resistance (MDR) (Nikaido 2009). Antimicrobial resistance contributes to unsuccessful management of bacterial pathogens, higher infection spread and perseverance (Tanwar *et al.* 2014). In the European Union, MDR infections are responsible for approx. 25,000 patient deaths per year, and result in extra healthcare costs and productivity losses in the EU reported to cost at least 1.5 billion euros each year (Department of Health 2016). The global threat of bacteria with MDR is alarming and there is a need for new therapeutic discoveries and improvement of existing infection control and antimicrobial practices.

Potential options for novel antimicrobials include the use of natural compounds such as those derived from plants (essential oils, phenolics, lectins and polyacetylenes), animals (lactoferrin, chitosan and lysozymes), bacteria (bacteriocin, reuterin), algae, and fungi (Cowan 1999, Gyawali and Ibrahim 2014). Recent approaches include exploring peptide based antibiotics (Roshan *et al.* 2018) and the sequencing of prokaryote genomes to discover novel antimicrobial molecules (Tracanna *et al.* 2017).

Essential oils (EOs) are compounds produced by plants, and are known to have activity against both Gram-negative and Gram-positive bacteria in both motile and sessile states (Millezi *et al.* 2016). EOs have been used in medicine for many years and are widely reported as possessing antibacterial, antifungal and anti-plasmodial properties (Utchariyakiat *et al.* 2016). However, of the ~3000 EOs known, only ~300 are used commercially (Ghabraie *et al.* 2016). Whilst it is thought that the majority of EOs act on the cell wall and membrane of bacteria (Faleiro 2011), studies assessing mode of action of individual oils are required. Furthermore, very few studies exploring the antimicrobial activities of EOs investigate their

potential for rapid killing, i.e. under 10 min contact time. Friedman *et al.* (2004) investigated the activities of EOs and their components against *E. coli* O157:H7 with incubation times of ≥5 min, and Tangjitjaroenkun *et al.* (2012) studied the antimicrobial effects of EO from *Zanthoxylum limonella* with incubation times of ≥3 min. However, no studies to date have reported rapid killing against *Ps. aeruginosa* using EOs.

Therefore, the purpose of this study was to assess the effects of a selection of EOs against a panel of known pathogenic bacteria. In addition, EOs exhibiting the greatest antimicrobial effects, were investigated for its rapid bactericidal potential and mode of action against *Ps. aeruginosa*.

MATERIALS AND METHODS

ESSENTIAL OILS (EOs)

Fifteen EOs were used, and included EOs from: bergamot (*Citrus bergamia*), cinnamon (*Cinnamomum zeylanicum*) bark and leaf, clove (*Eugenia caryophyllus*), grapefruit (*Citrus paradisi*), lavender (*Lavandula angustifolia*), lemon (*Citrus limonum*), lemongrass (*Cymbopogon citratus*), lime (*Citrus aurantifolia*), manuka (*Leptospermum scoparium*), sweet orange (*Citrus sinensis)*, rose geranium (*Pelargonium graveolens*), rosemary (*Rosmarinus officinalis*), rosewood (*Dalbergia sissoo*), tea tree (*Melaleuca alternifolia*) and thyme (*Thymus vulgaris*). All of these oils were donated by Amphora Aromatics Ltd, Bristol, UK for research purposes, with the exception of cinnamon leaf oil, which was sourced from Natural by Nature Ltd (Bedfordshire, UK). All the oils used in this study were extracted by steam distillation as indicated by the suppliers.

TEST ORGANISMS AND STANDARDISATION OF OVERNIGHT CULTURES

The bacteria tested were obtained from the microbiology culture collection at the University of the West of England, Bristol, UK. The strains used were: *Escherichia coli* (ATCC 23848), *Escherichia coli* (NCTC 9001), *Staphylococcus aureus* (ATCC 6358), *Staphylococcus aureus* (NCTC 12981), *Pseudomonas aeruginosa* (PAO1), *Pseudomonas aeruginosa* (NCTC 8505), Hospital Acquired Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolate, Methicillin-Susceptible *Staphylococcus aureus* (MSSA) (NCTC 13297), *Acinetobacter baumannii* (NCTC 12156) and *Acinetobacter baumannii* (ATCC 17978). The bacteria were stored on beads (Microbank, Pro Lab Diagnostics, Canada) at -80°C and revived on nutrient agar (NA; Oxoid, Hampshire, UK) slopes at 37°C for 24 h. Overnight broth cultures were prepared using one to three colonies of bacteria added to 10 mL of tryptone soy broth (TSB; Oxoid, Hampshire, UK) and incubated at 37°C for 24 h. Cultures were standardised by diluting with TSB and measuring OD at 600 nm to obtain a reading of 0.08-0.1 (McFarland 0.5), giving a standardised inoculum of ~1-2 x 10^8 CFU mL^{-1}.

DISC DIFFUSION ASSAY

Screening of EOs was performed using a paper disk diffusion approach adapted from the Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing (AST) Standards (Clinical and Laboratory Standards Institute 2015). Mueller-Hinton agar (MHA) plates (Oxoid, Hampshire, UK) were inoculated with 100 µL of standardised culture for each test bacterium, spread evenly over the entire surface of the agar using a sterile cotton swab by swabbing in three directions (Andrews 2007). The inoculum applied to each plate was ~1-2 x 10^7 CFU mL^{-1}. A volume of 10 µL of EO was used to saturate a set of six mm diameter filter paper discs (Whatman, Sigma Alrdich, UK), one of which was then placed onto the centre of each inoculated plate. Blank discs were used as a negative control and discs containing 30 µg gentamicin were used as a positive reference. Plates were incubated at

37°C for 24 h and antibacterial activity was assessed by measuring the diameter of the zones of inhibition in mm using Vernier callipers. Each experiment was repeated on three separate occasions, with a minimum of three replicates in each repeat experiment.

DETERMINATION OF MINIMAL INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The MIC was determined by the broth microdilution method as outlined by the CLSI (2012) and adapted from previously described methods (Cao *et al.* 2009). A 16% (v/v) stock solution of EO was prepared in TSB containing 10% (v/v) dimethylsulfoxide (DMSO; Sigma Alrdich, Dorset, UK) which is a solvent which serves a dispersal agent (Kačániová *et al.* 2017). Two-fold dilutions of this stock solution were made in TSB to produce a range of EO concentrations from 0.015% to 8% (v/v), with DMSO concentrations of 0.0098% to 5% (v/v) respectively. One hundred microliters of each EO concentration were added to wells of a 96-well microtitre plate. A standardised overnight culture was diluted 1/150 with TSB, and 100 µL of this was added to each well of the microtitre plate. Final concentrations of EO ranged from 0.007% to 4% (v/v) with DMSO concentrations of 0.0049% to 2.5% (v/v) respectively. Final cell density of the inoculum was approx. 5×10^5 CFU mL^{-1}. Microplate wells of EO dilutions without bacteria, and TSB and DMSO alone, were used as negative controls, and plates were incubated at 37°C for 24 h. MIC values were determined as the well with the lowest concentration of EO where no visible microbial growth was observed. Each concentration of EO had three replicates and was repeated four times.

MBC was determined by spot inoculation similar to methods outlined by Adukwu *et al.* (2012). Briefly, 10 µL was pipetted from the microtitre wells showing no growth onto MHA, and incubated for 24 h at 37°C. Each concentration of EO had three replicates and was repeated

four times. MBC values were determined as the lowest concentration of EO where no growth was observed.

GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) ANALYSIS

Following determination of MICs and MBCs, cinnamon bark EO demonstrated broad-spectrum activity against all tested organisms, including *Ps. aeruginosa* at <4% (v/v). Thus, this oil was selected for testing rapid bactericidal effects against *Ps. aeruginosa.*

GC-MS analysis of cinnamon bark EO was performed using methods adapted from Adukwu *et al.* (2012). Cinnamon leaf EO was also analysed as a comparator to the Bark EO to identify the differences in the components as these EOs are obtained from different parts of the same plant. GC-MS analysis used an Agilent 6890N Network Gas Chromatograph system and 5973 Network Mass Selective Detector (Agilent Technologies, USA). Samples were dissolved in acetone with a one µL injection volume. Inlet temperature was 300°C with a split ratio of 10:1. Temperature of transfer line was 300°C and solvent delay was three min. The carrier gas was helium with a column flow rate of one mL/min and operating at constant flow. The oven temperature started at 50°C and was held for two min, then increased until 280°C was reached at a rate of 10°C/min, with a total run time of 25 min. Compound separation was achieved with a HP-5MS column (30 m × 0.25 mm × 0.25 µm). An electron impact ion source at 230°C, and a quadrupole mass analyser at 150°C was used. Electron ionization mass spectrometric data were collected between 30 to 550 m/z. Oil components were identified by comparison of mass spectral patterns with those from the spectra from the National Institute of Standards and Technology (NIST) library (MS Search 2.0) provided by the software of the GC-MS system.

TIME-KILL ASSAY

Time kill kinetics were determined for cinnamon bark EO against *Ps. aeruginosa* PAO1 using methods adapted from Carson *et al.* (2002). Six 50 mL conical flasks containing 15 mL of TSB were inoculated with 50 µL of standardised *Ps. aeruginosa* PAO1 overnight culture to achieve approx. 5 x 10^5 CFU mL^{-1}. A 100 µL aliquot was removed from each flask and added to 9.9 mL of TSB, diluted serially and plated onto MHA to confirm starting inoculum density, representing the zero min time point (T0). Table one shows the range of volumes of EO and DMSO added to the flasks to achieve a series of concentrations for the time-kill assay. The concentration of cinnamon bark EO ranged from 0.125% (v/v; one x MIC) to 2% (v/v; 16 x MIC), with a negative control flask containing DMSO alone. After the addition of EO/DMSO, the flasks were vortexed and incubated stationary at room temperature. At each time point the flasks were vortexed again and aliquots were removed at two, four, six, 10 and 30 min and at one, two, four, six and 24 h, diluted serially and plated onto MHA. All plates were incubated at 37°C for 24 h before enumeration of the colonies. An antimicrobial compound is considered bactericidal if a ⩾99.9% decrease in the initial inoculum (i.e. a three-log reduction) is observed, as described by the CLSI (1999).

METABOLIC ACTIVITY ASSAY

A triphenyltetrazolium chloride (TTC) metabolic activity assay was performed to investigate the effect of EO on metabolic activity, as described by Ahmed (2013), during the time-kill assay. One hundred microlitre aliquots of the treated organisms from each time point were transferred to wells of a 96-well plate. A five µL volume of sterile 0.035 M aqueous solution of TTC (Sigma Alrdich, Dorset, UK) was added to each well and plates were wrapped in aluminium foil and incubated at 37 °C with orbital shaking at 120 rpm for 24 h. TSB containing no bacteria was used as a negative control. The presence of viable bacterial cells was indicated by the reduction of the yellow TTC to a red colour. OD of the wells was measured

using a TECAN Infinite® 200 PRO plate reader at 595 nm. Data were normalised to T0 and expressed as a percentage relevant to this time point. All assays were performed in triplicate on three separate occasions.

SCANNING ELECTRON MICROSCOPY (SEM)

SEM was used to assess morphological effects on *Ps. aeruginosa* PAO1 after treatment with cinnamon bark EO at 2% (v/v) for 10 min. Both untreated and treated samples were prepared for SEM using the method reported by Murtey and Ramasamy (2016) and adjusted as indicated below. Briefly, samples were allowed to settle on squares of poly-l-lysine coated microscope slides (Sigma Alrdich, Dorset, UK) in Petri dishes lined with damp filter paper for one h. The attached cells were fixed by immersion in 0.424 M glutaraldehyde (Sigma Alrdich, Dorset, UK) in 0.1 M phosphate buffer for 30 min, washed with the same buffer three times and then dehydrated through a series of increasing ethanol concentrations (35%, 50%, 75%, 95%, 95%, 100%, 100% v/v) for 10 min each, followed by hexamethyldisilizane (HMDS; Sigma Alrdich, Dorset, UK) for 10 min two times. The squares were mounted on aluminium stubs and coated with gold in a sputter coater and viewed using a scanning electron microscope (FEI Quanta 650 FEG, Sigma Aldrich, UK).

RELEASE OF NUCLEIC ACIDS AND PROTEINS

The release of 260 nm absorbing nucleic acids and 280nm absorbing proteins from *Ps. aeruginosa* PAO1 after treatment with cinnamon bark EO was measured according to methods described by Miksusanti *et al.* (2008), with some adaptations. A 50 mL conical flask containing 15 mL of cinnamon bark EO at either 2% (v/v) or 1.25% (v/v) in TSB with 1.25% (v/v) or 0.08% (v/v) of DMSO, respectively, was inoculated with 50 µL of standardised overnight *Ps. aeruginosa* PAO1 culture. The flask was vortexed for 30 s,

incubated at room temperature for two h and then a one mL aliquot was removed and filtered with 0.45 µm filter. The absorbance of this filtrate was measured at 260 nm and 280 nm using a Jenway 6305 UV-Vis spectrophotometer (Cole-Parmer, UK). Negative controls were treated with DMSO (1.25% v/v) alone. The absorbance of filtrate from controls without culture added were deducted from the absorbance of the respective samples with EO added. Experiments were performed in triplicate on three separate occasions and results are expressed as mean OD of nucleic acids (260 nm) and protein (280 nm).

DATA ANALYSIS

All data were analysed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA) and GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California, USA). An unpaired t-test was applied when the results of treated and untreated samples were assessed following the disc diffusion assay whilst the One-Way ANOVA and Dunnett's multiple comparison were used to assess any differences between treatments and control following the membrane permeability assay.

RESULTS

DISC DIFFUSION

The results from the antimicrobial susceptibility screening assay demonstrated that all the EOs used in this study were antimicrobial against most, if not all, the bacteria tested (Table 1). Three of the EOs, cinnamon bark, clove and thyme, produced zones of inhibition against all strains tested, including *Ps. aeruginosa* PAO1, demonstrating broad spectrum activity. Inhibition zones shown by thyme and cinnamon bark EO were significantly larger ($P<0.05$) than that of the gentamicin antibiotic reference in 70% and 60% of the strains used,

246 respectively. Thyme produced zones of inhibition ranging from 17.1 - 48.2 mm, whilst
247 cinnamon bark produced zones ranging from 16.7 – 36.2 mm. Rose geranium EO showed
248 activity against all strains excluding *Ps. aeruginosa* NCTC 8505, producing zones ranging
249 from 5.3 – 18.3 mm. Cinnamon leaf EO showed activity against all strains excluding *Ps.*
250 *aeruginosa* PAO1, producing zones ranging from 8.7 – 18.2 mm. Lavender, lemongrass,
251 lime, rosemary, rosewood and tea tree EOs showed effects against both Gram positive and
252 Gram negative bacteria, excluding the *Pseudomonas* species. Of these oils, lemongrass
253 produced the largest zones of inhibition, ranging from 10.1 - 36.9 mm. The smallest zones of
254 inhibition were produced by bergamot, grapefruit, lemon, manuka and sweet orange EOs.
255 Blank disc negative controls did not affect the growth of any of the strains. In accordance with
256 the breakpoint tables for inhibition zone interpretation published by the European Committee
257 on Antimicrobial Susceptibility Testing (EUCAST 2015), all bacteria in this study were
258 sensitive to gentamicin with the exception of MRSA and *Ac. baumannii* NCTC 12156.
259
260 DETERMINATION OF MIC AND MBC
261 Nine EOs were selected, based on their antimicrobial activity shown in the disc diffusion
262 assay, to determine their MIC and MBC (Table 2). The data show that rose geranium,
263 rosewood and tea tree produced MICs between 0.5 and 4% or greater (v/v) against most
264 strains and that MBC values for these are either equal or double the MIC value. Although
265 manuka EO demonstrated a MIC as low as 0.06% (v/v), the inhibitory effects were only
266 against Gram-positive *Staph. aureus* strains. Manuka EO did not demonstrate any
267 bactericidal action at <4% (v/v). Thyme, lemongrass, cinnamon leaf and clove EOs presented
268 MICs and MBCs of ≤1.0% (v/v) for all strains of bacteria excluding the *Ps. aeruginosa* strains.
269 The only EO which demonstrated broad spectrum antimicrobial activity in this investigation
270 was cinnamon bark EO. All the bacteria tested including the *Pseudomonas* spp. were

inhibited by cinnamon bark EO at ≤0.25% (v/v), which also demonstrated bactericidal activity at the same concentration.

GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) ANALYSIS

Components of cinnamon bark and cinnamon leaf EO and their percentage composition are reported in Table 3. There were noticeably more components from the cinnamon leaf EO than from cinnamon bark EO with 20 components identified from the leaf EO in comparison to 7 components identified from the bark EO. The major components from either EO were different and represented approx. 85% of the whole oil with the rest of the components representing the remaining 15% of the EO. For the Cinnamon bark EO, the main component was Σ-cinnamaldehyde representing approx. 85.312% of the EO whereas from the cinnamon leaf EO eugenol was identified as the major component representing 84.481% of the whole EO.

TIME-KILL ASSAY - CINNAMON BARK EO AGAINST *PS. AERUGINOSA* PAO1

A time-kill assay was performed to evaluate the bactericidal potential of cinnamon bark EO against *Ps. aeruginosa* PAO1 (Fig. 1). At the MIC, 0.125% (v/v), cinnamon bark EO was bactericidal (three-log reduction) against *Ps. aeruginosa* PAO1 after six h. At concentrations two-fold and four-fold of the MIC, 0.25% (v/v) and 0.5% (v/v), a 99.999% (five-log) kill, was achieved within 30 and six min respectively. At the higher EO concentrations of 1% and 2% (v/v), 5-log reduction was achieved within two min.

METABOLIC ACTIVITY ASSAY

Reduction of TTC from a yellow to red colour was observed in microtitre wells containing viable *Ps. aeruginosa*, but no colour change was observed when cells were non-viable (Fig.

296 2). Negative control wells with TSB containing no bacteria remained clear with no colour
297 change. All results obtained from the TTC assay reflected results from the time-kill assay
298 shown in Fig. 1. *Ps. aeruginosa* continued to show metabolic activity after 10 min of contact
299 time when cinnamon bark EO was at 0%, 0.125% and 0.25% (v/v), with some activity after
300 four min of contact time with EO at 0.5% (v/v), but no activity after six min. Metabolic activity
301 had ceased after two min of contact time at concentrations equal or greater to 1% (v/v).
302
303 SCANNING ELECTRON MICROSCOPY

304 Electron micrographs of both untreated and cinnamon bark EO treated *Ps. aeruginosa* cells
305 are presented in Figs 3-4. The untreated cells (Figs 3a and 3b) show a turgid structure with
306 a particulate surface, and are of expected lengths (one to five µm). In contrast, cells treated
307 with cinnamon bark EO showed an altered morphology (Figs 4a and 4b), with cells appearing
308 collapsed with loss of turgidity and few surface particles after 10 min contact time with 2%
309 (v/v) cinnamon bark EO.
310
311 RELEASE OF NUCLEIC ACIDS AND PROTEINS

312 Relative OD of filtrate from *Ps. aeruginosa* untreated or treated with cinnamon bark EO
313 measured at 260nm and 280nm are displayed in Figs 5a and 5b, respectively. Increased
314 OD_{260nm} readings signifies an increase in nucleic acids, whilst an increase in OD_{280nm}
315 indicates an increase in proteins (Miksusanti *et al.* 2008) released from bacterial cells. An
316 increase was seen in both OD_{260nm} and OD_{280nm} when bacteria were treated with cinnamon
317 bark EO at either 0.125% or 2% (v/v) when compared to the untreated cultures. There was
318 a significant difference ($P \leq 0.01$) between readings for the untreated bacteria and bacteria
319 treated with 2% (v/v) EO.
320

DISCUSSION

This study examined the effect of EOs against pathogenic bacteria, some of which exhibit antimicrobial resistance. Disc diffusion assays using EOs at 100% (v/v) concentration demonstrated antimicrobial activity against the selected panel of Gram-positive and Gram-negative bacteria. The use of an established antibiotic (gentamicin) provided a reference against which the sensitivity of the bacteria was assessed. Gentamicin sensitivity was observed in all tested bacteria with the exception of MRSA and *Ac. baumannii* NCTC 12156. Zones of inhibition breakpoints for MRSA and *Ac. baumannii* treated with gentamicin are 18 mm and 17 mm, respectively.

The disc diffusion assay showed that the oils showing the greatest inhibitory effects were thyme, cinnamon bark and lemongrass. Of these, thyme EO was most effective, an observation reported previously by Semeniuc *et al.* (2017) where it was most effective when compared to three other oils. Although the MRSA strain used was resistant to gentamicin in this study, cinnamon bark, lemongrass, manuka, rosemary and thyme EOs all produced zones greater than 18 mm. *Ac. baumannii* NCTC 12156 also showed resistance against gentamicin, although tea tree, thyme, rosewood, clove, cinnamon bark and cinnamon leaf EOs all produced zones ≥ 17 mm. These data are in line with published reports (Doran *et al.* 2009, Adukwu *et al.* 2012, Priti and Shridhar 2012, Yap *et al.* 2014, Sakkas *et al.* 2016), which indicate that EOs are capable of working effectively against bacteria resistant to commercial antibiotics.

Lemon, lemongrass, manuka, sweet orange and tea tree EO were more effective against the Gram-positive bacteria in comparison to the Gram-negative bacteria. Lemongrass and manuka in particular were shown to have lower MICs/MBCs for *Staph. aureus* when compared to other bacteria tested. These findings are in agreement with studies that found manuka EO to be more effective against *Staph. aureus* compared to *E. coli* and *Ps.*

346 *aeruginosa* (Porter and Wilkins 1998), and studies where lemongrass EO also showed good
347 effect against Gram-positive bacteria (Hammer *et al.* 1999). In the present study, cinnamon
348 bark at very low concentrations (i.e. 0.015% v/v), was bactericidal against MRSA, a finding
349 which is supported by Cui *et al.* (2016), where cinnamon oil was found to be bactericidal
350 against MRSA at 0.2 µg mL^{-1}.
351 Cinnamon leaf, clove, lemongrass, rosewood and thyme oils at <4% (v/v) were bactericidal
352 against all the bacteria, excluding *Pseudomonas* species. A study by Kavanaugh and
353 Ribbeck (2012) also reported a lack of bactericidal activity from clove, thyme and tea tree EO
354 against *Ps. aeruginosa* spp. at concentrations less than or equal to 4% (v/v). Different studies
355 have shown that EOs are more effective against Gram-positive bacteria (Lodhia *et al.* 2009),
356 which is supported by some of the results presented here. However, equal or greater efficacy
357 was observed when some of the oils were exposed to the Gram-negative bacteria. Bergamot,
358 cinnamon bark, cinnamon leaf, clove, grapefruit, lime, rose geranium and rosewood all
359 produced greater zones of inhibition in some Gram-negative bacteria compared to zones
360 produced for Gram-positive bacteria. Of the oils tested for MIC, cinnamon leaf, clove,
361 rosewood, tea tree and thyme had lower or equal MIC for Gram-negative bacteria. Cinnamon
362 bark EO demonstrated bactericidal effects against all the strains used in this study at very
363 low concentrations (i.e. ≤ 0.25% v/v).
364 Cinnamon bark EO was thus selected for further investigation due to the broad-spectrum
365 activity demonstrated against all the bacteria species tested and as it was the only oil to exert
366 antimicrobial activity against the two strains of *Ps aeruginosa* during the antimicrobial
367 screening phase.
368 GC-MS analysis showed that the major component of cinnamon leaf oil used in this study
369 was eugenol, which was 84.5% of the total oil composition. This is similar to findings in the
370 current literature (Mallavarapu *et al.* 1995, Paranagama *et al.* 2001, Raina *et al.* 2001,

371 Jayawardena and Smith 2010). In contrast, the major component of the cinnamon bark oil
372 used in this study was Σ-cinnamaldehyde at 85.3% of the total composition. The high levels
373 of this component in the bark EO has been demonstrated elsewhere in other studies
374 (Paranagama *et al.* 2001, Jayawardena and Smith 2010, Shahina *et al.* 2018). This
375 contrasting composition of the two oils provides an explanation of their different antimicrobial
376 effects, and it has been shown that cinnamaldehyde has a superior antimicrobial effect when
377 compared to eugenol (López *et al.* 2007, Sanla-Ead *et al.* 2011, Brnawi *et al.* 2018). The
378 differences seen in oil composition also highlights the importance of adequate analysis of
379 EOs, using methods such as GC-MS, to confirm the origin of the oil and identify which part
380 of the plant it is derived from.
381 From the time-kill assay in the present study, cinnamon bark EO exhibited bactericidal effects
382 with rapid killing action (three-log reduction) at ≤30 min at concentrations ≥0.25% (v/v). These
383 findings are further evidence of the broad-spectrum potential of EOs, against both Gram-
384 negative and Gram-positive bacteria. The TTC assay was used to indicate the presence or
385 absence of metabolically active cells which would convert TTC to a coloured formazan
386 derivative (Sabaeifard *et al.* 2014). This assay is an important indicator for bacterial viability,
387 as the intensity of the colour measured is directly proportional to the number of living cells
388 (Moussa et al. 2013). The results obtained correlate well with the time-kill assay. The assay
389 provides evidence for the possible mode of action of cinnamon bark EO, suggesting loss of
390 metabolic function in the *Ps. aeruginosa* strain following exposure. These findings indicate
391 that the respiratory activity necessary to reduce TTC was not present, which could also
392 indicate a loss of membrane-selective permeability and loss of other vital enzymatic actions,
393 as proposed by Bouhdid *et al.* (2010). However, it has been reported that TTC reduction may
394 not correlate exactly with MIC, and end-points are less easily determined using the TTC

assay when compared to that of other redox indicators, such as resazurin (Mann and Markham 1998).

The bactericidal effect of cinnamon bark EO on *Ps. aeruginosa* cell morphology was examined by scanning electron microscopy (SEM). After only 10 min of exposure to the EO, morphological changes to the treated cells were pronounced, and suggested total cell collapse and likely loss of viability. Reports on the mechanism of action of EOs suggest their role in the destruction of the cell wall, damage to the cytoplasmic membrane and membrane proteins, and cell leakage (Goldbeck *et al.* 2014). This suggestion is supported by data from the membrane permeability assay, which indicate that cinnamon bark EO causes leakage of nucleic acids, a probable result of a compromised cell membrane. This is in agreement with Bouhdid *et al.* (2010) who found that cinnamon bark oil affected the membrane of *Ps. aeruginosa* which ultimately led to cell death. This contrasts with the findings by Cox and Markham (2007) and Helander *et al.* (1998) who showed that Σ-cinnamaldehyde, a predominant component of the cinnamon bark EO used in this study, did not have an effect on the membrane of *Ps. aeruginosa* and was deemed to not be a pore forming compound. This highlights the importance of using whole oils, and not their isolated compounds, as it may not reflect the antimicrobial capability of an oil. It also further highlights the need for oil analysis to identify the blend of different compounds present and the role they play in the antimicrobial effects of an EO.

Cinnamon EO is sourced from either the bark or the leaf of the plants (Park *et al.* 2018), with the EO extracted from bark most often investigated for its antimicrobial activity (Nabavi *et al.* 2015). Cinnamon bark EO is already well described for its antimicrobial activity (Manso *et al.* 2014, Wu *et al.* 2015, Wen *et al.* 2016, Zhang *et al.* 2016, Chouhan *et al.* 2017), and shown to be highly bactericidal against *Pseudomonas* spp. (Utchariyakiat *et al.* 2016). However, this

study is the first to demonstrate to our knowledge the rapid killing effect of the cinnamon bark EO against *Ps. aeruginosa,* with contact times of <30 min.

In summary, this study has shown that EOs are effective antimicrobial agents and many possess broad-spectrum activity. Cinnamon bark EO in particular has demonstrated strong bacteriostatic and bactericidal action against *Ps. aeruginosa*. This provides a sound basis for further work to explore the use of cinnamon bark EO as an alternative to conventional antimicrobial products owing to its fast-acting bactericidal properties at low concentrations.

The present study has confirmed that cinnamon bark is an antimicrobial EO which provides broad spectrum, high bactericidal activity when used at low concentrations and within a short contact time. These qualities make it an ideal candidate as an alternative to current antimicrobials, for example on surfaces in clinical and food preparation applications, in disinfection and infection control. Many studies investigating time kill and bactericidal activity of EOs expose bacteria to longer contact times, though it is important that shorter contact times are investigated and this study highlights the importance of performing antimicrobial tests using contact times <10 min.

Findings from this study further support the reports that mode of action of cinnamon bark EO against *Ps. aeruginosa* is due to effects against the membrane of the bacteria, leading to cell leakage and cell death, shown here in the results of SEM and 260/280nm absorbance assay.

Although EOs are generally recognised as safe (GRAS) by the Food and Drugs Administration (FDA), including that of cinnamon (Maisanaba *et al.* 2017), more investigation is needed to assess cinnamon EO's application to control bacterial pathogens and subsequent interaction with humans. This study also highlights the importance of analysing these natural products before use, in order to determine their composition and to identify their key components. The composition and antimicrobial diversity observed amongst oils

originating from different parts of the same species of plant emphasizes the need for batch control and consistency when developing these oils for antimicrobial purposes.

ACKNOWLEDGEMENTS

This work was supported by Medical Wire and Equipment (MWE) and the Faculty of Health and Applied Sciences, University of the West of England, Bristol via joint funding. EOs were kindly donated by Amphora Aromatics, Bristol. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

CONFLICT OF INTEREST

No conflict of interest declared.

REFERENCES

Adukwu, E.C., Allen, S.C.H., and Phillips, C.A., 2012. The anti-biofilm activity of lemongrass (*Cymbopogon flexuosus*) and grapefruit (*Citrus paradisi*) essential oils against five strains of *Staphylococcus aureus*. *J Appl Microbiol*, 113, 1217–1227.

Ahmed, M.N.A., 2013. *Effects of Escapin Intermediate Products (EIP-K) on Biofilms of Pseudomonas aeruginosa.* MSc. Thesis. Georgia State University.

Andrews, J.M., 2007. BSAC standardized disc susceptibility testing method (version 6). *J Antimicrob Chemother,* 60(1), 20–41.

Bouhdid, S., Abrini, J., Amensour, M., Zhiri, A., Espuny, M.J., and Manresa, A., 2010. Functional and ultrastructural changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Cinnamomum verum* essential oil. *J Appl Microbiol*, 109(4), 1139–1149.

Brnawi, W.I., Hettiarachchy, N.S., Horax, R., Kumar-Phillips, G., Seo, H.-S., and Marcy, J., 2018. Comparison of cinnamon essential oils from leaf and bark with respect to antimicrobial activity and sensory acceptability in strawberry shake. *J Food Sci*, 83(2), 475–480.

469 Cao, L., Si, J.Y., Liu, Y., Sun, H., Jin, W., Li, Z., Zhao, X.H., and Pan, R. Le, 2009. Essential oil
470 composition, antimicrobial and antioxidant properties of *Mosla chinensis* Maxim. *Food Chem,*
471 115(3), 801–805.
472 Carson, C.F., Mee, B.J., and Riley, T. V, 2002. Mechanism of action of *Melaleuca alternifolia* (tea
473 tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance
474 assays and electron microscopy. *Antimicrob Agents Chemother*, 46(6), 1914–20.
475 Chouhan, S., Sharma, K., and Guleria, S., 2017. Antimicrobial activity of some essential oils -
476 present status and future perspectives. *Medicines*, 4 (58), pp. 1-21.
477 Clinical and Laboratory Standards Institute, 1999. *M26-A: Methods for determining bactericidal*
478 *activity of antimicrobial agents; approved guideline.* Pennsylvania, USA: CLSI.
479 Clinical and Laboratory Standards Institute, 2012. *M07-A9: Methods for dilution antimicrobial*
480 *susceptibility tests for bacteria that grow aerobically; Approved standard—ninth edition.*
481 Pennsylvania, USA: CLSI.
482 Clinical and Laboratory Standards Institute, 2015. *M02-A12: Performance standards for*
483 *antimicrobial disk susceptibility tests; approved standard—twelfth edition.* Pennsylvania, USA:
484 CLSI.
485 Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clin Microbiol Rev*, 12(4), 564–82.
486 Cox, S.D. and Markham, J.L., 2007. Susceptibility and intrinsic tolerance of *Pseudomonas*
487 *aeruginosa* to selected plant volatile compounds. *J Appl Microbiol*, 103(4), 930–936.
488 Cui, H., Li, W., Li, C., Vittayapadung, S., and Lin, L., 2016. Liposome containing cinnamon oil with
489 antibacterial activity against methicillin-resistant *Staphylococcus aureus* biofilm. *Biofouling*,
490 32(2), 215–225.
491 Department of Health, 2016. Antimicrobial resistance empirical and statistical evidence-base. *Public*
492 *Health England*, (September),1–63
493 Doran, A.L., Morden, W.E., Dunn, K., and Edwards-Jones, V., 2009. Vapour-phase activities of
494 essential oils against antibiotic sensitive and resistant bacteria including MRSA. *Lett Appl*
495 *Microbiol*, 48(4), 387–392.
496 EUCAST, 2015. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for

497 interpretation of MICs and zone diameters. *The European Committee on Antimicrobial*
498 *Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version*
499 *8.0, 2018. http://www.eucast.org.*, 0-77.
500 Faleiro, M., 2011. 'The mode of antibacterial action of essential oils' in Méndez-Vilas A. (ed.)
501 *Science against microbial pathogens: communicating current research and technological*
502 *advances.* World Scientific Publishing Co. Pte. Ltd: London, 1143-1156
503 Friedman, M., Henika, P.R., Levin, C.E., and Mandrell, R.E., 2004. Antibacterial activities of plant
504 essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica*
505 in apple juice. *J Agric Food Chem*, 52(19), 6042–6048.
506 Ghabraie, M., Vu, K.D., Tata, L., Salmieri, S., and Lacroix, M., 2016. Antimicrobial effect of essential
507 oils in combinations against five bacteria and their effect on sensorial quality of ground meat.
508 *LWT - Food Science and Technology*, 66, 332–339.
509 Goldbeck, J.C., Victoria, F.N., Motta, A., Savegnago, L., Jacob, R.G., Perin, G., Ao, J.~, Ao, L.~,
510 Padilha, W., and Silva, D., 2014. Bioactivity and morphological changes of bacterial cells after
511 exposure to 3-(p-chlorophenyl)thio citronellal. *LWT - Food Science and Technology*, 59(2),
512 813–819.
513 Gyawali, R. and Ibrahim, S.A., 2014. Natural products as antimicrobial agents. *Food Control*, 46,
514 412–429.
515 Hammer, K.A., Carson, C.F., and Riley, T. V, 1999. Antimicrobial activity of essential oils and other
516 plant extracts. *J Appl Microbiol*, 86(6), 985–90.
517 Helander,I.M., Alakomi,H-L,Latva-Kala,K., Mattila-Sandholm, T., Pol, I, Smid, E.J., Gorris, L.G.M.,
518 and Wright, A., 1998. Characterization of the Action of Selected Essential Oil Components on
519 Gram-Negative Bacteria. *J Agric Food Chem*, 46 (9), 3590–3595
520 Jayawardena, B. and Smith, R.M., 2010. Superheated water extraction of essential oils from
521 *Cinnamomum zeylanicum* (L.). *Phytochem Anal*, 21(5), 470–472.
522 Kačániová, M., Terentjeva, M., Vukovic, N., Puchalski, C., Roychoudhury, S., Kunová, S., Klūga, A.,
523 Tokár, M., Kluz, M., and Ivanišová, E., 2017. The antioxidant and antimicrobial activity of
524 essential oils against *Pseudomonas* spp. isolated from fish. *Saudi Pharmaceutical Journal*,

25(8), 1108–1116.

Kavanaugh, N.L. and Ribbeck, K., 2012. Selected antimicrobial essential oils eradicate *Pseudomonas* spp. and *Staphylococcus aureus* biofilms. *Appl Environ Microbiol*, 78(11), 4057–61.

Lodhia, M.H., Bhatt, K.R., and Thaker, V.S., 2009. Antibacterial activity of essential oils from palmarosa, evening primrose, lavender and tuberose. *Indian Journal of Pharmaceutical Sciences*, 71(2), 134–6.

López, P., Sánchez, C., Batlle, R., and Nerín, C., 2007. Vapor-phase activities of cinnamon, thyme, and oregano essential oils and key constituents against foodborne microorganisms. *J Agric Food Chem*, 55(11), 4348–4356.

Maisanaba, S., Llana-Ruiz-Cabello, M., Gutiérrez-Praena, D., Pichardo, S., Puerto, M., Prieto, A.I., Jos, A., and Cameán, A.M., 2017. New advances in active packaging incorporated with essential oils or their main components for food preservation. *Food Reviews International*, 33, 447–515.

Mallavarapu, G.R., Ramesh, S., Chandrasekhara, R.S., Rajeswara Rao, B.R., Kaul, P.N., and Bhattacharya, A.K., 1995. Investigation of the essential oil of cinnamon leaf grown at Bangalore and Hyderabad. *Flavour Fragr J*, 10, 239–242.

Mann, C.M. and Markham, J.L., 1998. A new method for determining the minimum inhibitory concentration of essential oils. *J Appl Microbiol*, 84(4), 538–544.

Manso, S., Pezo, D., Gómez-Lus, R., and Nerín, C., 2014. Diminution of aflatoxin B1 production caused by an active packaging containing cinnamon essential oil. *Food Control*, 45, 101–108.

Miksusanti, M., Sri Laksmi Jenie, B., Pontjo Priosoeryanto, B., Syarief, R., and Gatot, T.R., 2008. Mode of action temu kunci (*kaempferia pandurata*) essential oil on *e. coli* k1.1 cell determined by leakage of material cell and salt tolerance assays. *HAYATI Journal of Biosciences*, 15, 56–60.

Millezi, A.F., Piccoli, R.H., Oliveira, J.M., and Pereira, M.O., 2016. Anti-biofim and antibacterial effect of essential oils and their major compounds. *Journal of Essential Oil Bearing Plants*, 19, 624–631.

553 Moussa, S.H., Tayel, A.A., Al-Hassan, A.A., and Farouk, A., 2013. Tetrazolium/Formazan Test as
554 an Efficient Method to Determine Fungal Chitosan Antimicrobial Activity. *Journal of Mycology*, 2013,
555 1–7.
556 Murtey, M. Das and Ramasamy, P., 2016. Sample preparations for scanning electron microscopy –
557 Life sciences. In: M. Janecek, ed. *Modern Electron Microscopy in Physical and Life Sciences*.
558 InTech, 161–185.
559 Nabavi, S.F., Di Lorenzo, A., Izadi, M., Sobarzo-Sánchez, E., Daglia, M., and Nabavi, S.M., 2015.
560 Antibacterial Effects of Cinnamon: From Farm to Food, Cosmetic and Pharmaceutical
561 Industries. *Nutrients*, 7, 7729–48.
562 Nikaido, H., 2009. Multidrug Resistance in Bacteria. *Annual Review of Biochemistry*, 78, 119–146.
563 Paranagama, P.A., Wimalasena, S., Jayatilake, G.S., Jayawardena, A.L., Senanayake, U.M., and
564 Mubarak, A.M., 2001. A comparison of essential oil constituents of bark, leaf, root and fruit of
565 cinnamon (*Cinnamomum zeylanicum* Blume) grown in Sri Lanka. *J Natl Sci Found*, 29, 147–
566 153.
567 Park, J.B., Kang, J.H., and Song, K. Bin, 2018. Antibacterial activities of a cinnamon essential oil
568 with cetylpyridinium chloride emulsion against *Escherichia coli* O157:H7 and *Salmonella*
569 *Typhimurium* in basil leaves. *Food Sci Biotechnol*, 27, 47–55.
570 Porter, N.G. and Wilkins, A.L., 1998. Chemical, physical and antimicrobial properties of essential
571 oils of *Leptospermum scoparium* and *Kunzea ericoides*. *Phytochemistry*, 50, 407–415.
572 Priti, V. and Shridhar, P., 2012. Effect of essential oils on MDR pathogens: a comparative study.
573 *Journal of Environmental Research and Development*, 6, 462–466.
574 Raina, V.K., Srivastava, S.K., Aggarwal, K.K., Ramesh, S., and Kumar, S., 2001. Essential oil
575 composition of *Cinnamomum zeylanicum* Blume leaves from Little Andaman, India.
576 *Flavour Fragr J*, 16, 374–376.
577 Roshan, N., Hammer, K.A., and Riley, T. V., 2018. Non-conventional antimicrobial and alternative
578 therapies for the treatment of *Clostridium difficile* infection. *Anaerobe*, 49, 103–111.
579 Sabaeifard, P., Abdi-Ali, A., Soudi, M.R., and Dinarvand, R., 2014. Optimization of tetrazolium salt
580 assay for *Pseudomonas aeruginosa* biofilm using microtiter plate method. *J Microbiol Methods,*

105, 134–140.

Sakkas, H., Gousia, P., Economou, V., Sakkas, V., Petsios, S., and Papadopoulou, C., 2016. *In vitro* antimicrobial activity of five essential oils on multidrug resistant Gram-negative clinical isolates. *J Intercult Ethnopharmacol*, 5, 212–8.

Sanla-Ead, N., Jangchud, A., Chonhenchob, V., and Suppakul, P., 2011. Antimicrobial activity of cinnamaldehyde and eugenol and their activity after incorporation into cellulose-based packaging films. *Packag Technol Sci*, 25, 7–17.

Semeniuc, C.A., Pop, C.R., and Rotar, A.M., 2017. Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *J Food Drug Anal*, 25, 403–408.

Shahina, Z., El-Ganiny, A.M., Minion, J., Whiteway, M., Sultana, T., and Dahms, T.E.S., 2018. *Cinnamomum zeylanicum* bark essential oil induces cell wall remodelling and spindle defects in *Candida albicans*. *Fungal Biol Biotechnol*, 5(3), 1-16.

Tangjitjaroenkun, J., Chavasiri, W., Thunyaharn, S., and Yompakdee, C., 2012. Bactericidal effects and time-kill studies of the essential oil from the fruits of *Zanthoxylum limonella* on multi-drug resistant bacteria. *Journal of Essential Oil Research*, 24, 363–370.

Tanwar, J., Das, S., Fatima, Z., and Hameed, S., 2014. Multidrug resistance: an emerging crisis. *Interdisciplinary Perspectives on Infectious Diseases,* 2014, 1-7.

Tracanna, V., de Jong, A., Medema, M.H., and Kuipers, O.P., 2017. Mining prokaryotes for antimicrobial compounds: from diversity to function. *FEMS Microbiol Rev*, 41, 417–429.

Utchariyakiat, I., Surassmo, S., Jaturanpinyo, M., Khuntayaporn, P., and Chomnawang, M.T., 2016. Efficacy of cinnamon bark oil and cinnamaldehyde on anti-multidrug resistant *Pseudomonas aeruginosa* and the synergistic effects in combination with other antimicrobial agents. *BMC Complement Altern Med*, 16(158), 1-7.

Wen, P., Zhu, D.-H., Wu, H., Zong, M.-H., Jing, Y.-R., and Han, S.-Y., 2016. Encapsulation of cinnamon essential oil in electrospun nanofibrous film for active food packaging. *Food Control*, 59, 366–376.

Wu, J., Liu, H., Ge, S., Wang, S., Qin, Z., Chen, L., Zheng, Q., Liu, Q., and Zhang, Q., 2015. The

preparation, characterization, antimicrobial stability and *in vitro* release evaluation of fish gelatin films incorporated with cinnamon essential oil nanoliposomes. *Food Hydrocolloids*, 43, 427–435.

Yap, P.S.X., Yiap, B.C., Ping, H.C., and Lim, S.H.E., 2014. Essential oils, a new horizon in combating bacterial antibiotic resistance. *Open Microbiol J*, 8, 6–14.

Zhang, Y., Liu, X., Wang, Y., Jiang, P., and Quek, S., 2016. Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. *Food Control*, 59, 282–289.

List of Figures

List of Tables

650 Table 1 - Antimicrobial activity of fifteen essential oils using disc diffusion assay. Values are mean inhibition zone (mm) ± SE of 9
651 replicates (n=3).

Essential Oil	*E. coli* B ATCC 23848	*E. coli* NCTC 9001	*Staph. aureus* ATCC 6358	*Staph. aureus* NCTC 12981	Hospital Acquired MRSA isolate	MSSA NCTC 13297	*Ps. aeruginosa* PAO1	*Ps. aeruginosa* NCTC 8505	*Ac. baumannii* NCTC 12156	*Ac. baumannii* ATCC 17978
Bergamot	11.0±0.4†	-	7.0±0.0	10.7±0.4	6.4±1.6	5.0±1.3	-	-	5.1±1.3	4.7±1.2
Cinnamon Bark	36.2±1.2†	26.6±0.7†	30.8±0.5†	28.7±0.5	35.4±1.8†	27.7±1.1	17.3±0.7	16.7±1.4	28.6±0.7†	24.7±0.8†
Cinnamon Leaf	18.2±0.4	13.2±0.2	15.4±0.2	17.9±0.7	14.8±0.2	12.9±0.3	-	8.7±0.3	17.9±0.2	16.0±0.0
Clove	20.4±0.6	13.2±0.4	15.0±0.7	20.1±0.8	15.3±1.1	13.3±0.1	5.2±1.3	5.2±1.3	18.1±0.4†	15.8±0.5
Grapefruit	13.6±0.5	8.5±1.4	9.8±0.3	11.2±0.7	11.0±0.4	10.3±0.7	-	-	2.3±1.2	-
Lavender	12.3±0.7	7.7±0.3	14.7±1.7	12.1±0.4	11.6±1.0	9.8±0.2	-	-	8.9±0.5	9.1±0.3
Lemon	2.9±1.0	-	9.0±0.9	6.4±0.7	6.2±0.8	4.7±2.1	-	-	-	-
Lemongrass	32.6±1.0†	10.1±0.2	29.2±0.9	27.6±0.7	36.9±0.8†	26.8±2.1	-	-	14.2±1.5	24.6±2.6
Lime	14.7±0.8	10.6±1.1	11.1±0.3	12.8±0.6	10.1±0.8	10.1±0.7	-	-	12.0±0.6	11.6±0.2
Manuka	7.3±0.3	5.3±1.5	19.7±4.0	20.9±3.9	21.6±4.7	17.3±3.7	-	-	5.0±1.2	-
Sweet Orange	9.1±1.9	3.0±1.3	11.3±3.2	7.9±3.5	9.3±5.4	7.4±4.3	-	-	2.3±1.3	-
Rose Geranium	18.3±0.3	11.3±0.3	17.7±0.6	15.5±0.8	12.3±0.4	13.4±0.3	5.3±1.0	-	8.6±0.2	11.4±0.6
Rosemary	15.6±1.2	16.1±0.3	16.8±0.7	19.5±2.1	19.2±1.4†	17.4±1.1	-	-	16.5±1.3	14.7±1.4
Rosewood	21.7±2.0	14.7±0.7	16.4±2.1	20.3±1.0	16.2±0.4	14.0±0.7	-	-	20.04±1.1†	16.6±0.5
Tea Tree	16.7±0.6	16.4±0.6	23.4±1.7	22.4±2.1	17.2±0.5	13.5±1.0	-	-	18.9±1.2†	16.6±0.7
Thyme	46.2±4.8†	37.2±1.1†	48.2±2.1†	36.4±2.2	35.4±3.0†	35.8±2.8†	21.2±2.0	17.1±4.5	41.6±2.1†	36.4±5.3†
Gentamicin*	24.4±0.2	22.7±0.2	27.7±0.2	35.3±0.2	12.4±0.2	26.4±0.2	20.3±0.2	26.0±0.0	12.1±0.1	19.6±0.2

652 * gentamicin disc (30 µg)
653 - no activity
654 † value is significantly greater than gentamicin positive reference (P<0.05)

655 Table 2 - The antimicrobial activity of eight essential oils, using the broth microdilution method to find minimal inhibitory
656 concentration and minimum bactericidal concentration (% v/v) of 12 replicates (n=4).

657

Essential Oil	*E. coli B* ATCC 23848		*E. coli* NCTC 9001		*Staph. aureus* ATCC 6358		*Staph. aureus* NCTC 12981		Hospital Acquired MRSA isolate		MSSA NCTC 13297		*Ps. aeruginosa* PAO1		*Ps. aeruginosa* NCTC 8505		*Ac. baumannii* NCTC 12156		*Ac. baumannii* ATCC 17978	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Cinnamon Bark	0.03	0.125	0.06	0.125	0.06	0.125	0.03	0.06	0.015	0.015	0.03	0.125	0.125	0.125	0.25	0.25	0.03	0.125	0.06	0.125
Cinnamon Leaf	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.25	0.125	0.25	>4.0	>4.0	>4.0	>4.0	0.125	0.125	0.125	0.125
Clove	0.125	0.125	0.125	0.125	0.125	0.25	0.125	0.25	0.25	0.25	0.25	0.25	>4.0	>4.0	>4.0	>4.0	0.125	0.125	0.125	0.125
Lemongrass	0.25	0.25	1.0	1.0	0.25	0.25	0.25	0.25	0.12	0.25	0.25	0.25	>4.0	>4.0	>4.0	>4.0	1.0	1.0	0.5	1.0
Manuka	>4.0	>4.0	>4.0	>4.0	0.06	>4.0	0.06	>4.0	0.06	>4.0	0.06	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0
Rose Geranium	1.0	2.0	>4.0	>4.0	0.5	1.0	1.0	2.0	1.0	2.0	1.0	2.0	>4.0	>4.0	>4.0	>4.0	2.0	4.0	>4.0	>4.0
Rosewood	0.5	1.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0	>4.0	>4.0	>4.0	>4.0	0.5	1.0	1.0	1.0
Tea tree	1.0	1.0	1.0	1.0	2.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	4.0	4.0	2.0	2.0
Thyme	0.125	0.25	0.25	0.25	0.125	0.50	0.25	0.25	0.25	0.50	0.25	0.50	>4.0	>4.0	>4.0	>4.0	0.125	0.125	0.25	0.25

658

659 Table 3 - Percentage (%) composition of components within cinnamon bark and
660 cinnamon leaf essential oil after gas chromatography mass spectrometry (GCMS)
661 analysis.

Component	**Percentage (%) of total composition of oil**	
	Cinnamon Bark	**Cinnamon Leaf**
Σ-Cinnamaldehyde	85.312	0.185
D-limonene	4.665	-
Eucalyptol	3.823	0.160
Eugenol	3.366	84.481
Benzyl benzoate	1.935	2.752
β-linalool	0.897	1.627
Caryophyllene	-	3.224
Acetyleugenol	-	2.191
α-pinene	-	0.982
Camphene	-	0.282
β-terpinene	-	0.242
α-phellandrene	-	0.957
3-carene	-	0.04
Isoterpinolene	-	0.062
m-cymene	-	0.638
2-thujene	-	0.201
p-menth-1-en-8-ol	-	0.122
Copanene	-	0.633
Cinnamylalcohol acetate	-	0.640
α-caryophyllene	-	0.434
Carypyllene oxide	-	0.141
Total	99.998	99.994

662