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Characterisation of Antimicrobial Extracts from Dandelion Root (*Taraxacum officinale*) Using LC-SPE-NMR

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Plant extracts have traditionally been used as sources of natural antimicrobial compounds, although in many cases, the compounds responsible for their antimicrobial efficacy have not been identified. In this study, crude and dialysed extracts from dandelion root (*Taraxacum officinale*) were evaluated for their antimicrobial properties against Gram positive and Gram negative bacterial strains. The methanol hydrophobic crude extract (DRE3) demonstrated the strongest inhibition of microbial growth against *Staphylococcus aureus*, methicillin-resistant *S. aureus* and *Bacillus cereus* strains. Normal phase (NP) fractionation of DRE3 resulted in two fractions (NPF4 and NPF5) with enhanced antimicrobial activity. Further NP fractionation of NPF4 resulted in two fractions (NPF403 and NPF406) with increased antimicrobial activity. Further isolation and characterisation of compounds in NPF406 using liquid chromatography solid phase extraction nuclear magnetic resonance LC-SPE-NMR resulted in the identification of 9-hydroxyoctadecatrienoic acid and 9-hydroxyoctadecadienoic acid, while the phenolic compounds vanillin, coniferaldehyde and *p*-methoxyphenylglyoxylic acid were also identified respectively. The molecular mass of these compounds was confirmed by LC mass spectroscopy (MS)/MS. In summary, the antimicrobial efficacy of dandelion root extracts demonstrated in this study support the use of dandelion root as a source of natural antimicrobial compounds. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: Taraxacum officinale; antimicrobial; minimum inhibitory concentration; flash chromatography; LC-MS/MS; LC-SPE-NMR.

Abbreviations: 9-HODE, 9-hydroxyoctadecadienoic; 9-HOTE, 9-hydroxyoctadecatrienoic; COSY, correlation spectroscopy; CD_3CN, deuterated acetonitrile; DCM, dichloromethane; DMSO, dimethyl sulphoxide; DAD, diode array detector; ESI, electrospray ionisation; INT, iodonitrotetrazolium chloride; LC-MS, liquid chromatography mass spectroscopy; LC-SPE-NMR, liquid chromatography solid phase extraction nuclear magnetic resonance; $MgSO_4$, magnesium sulphate; MRSA, methicillin-resistant *Staphylococcus aureus*; MIC, minimum inhibitory concentration; MWCO, molecular weight cut-off; NP, normal phase; Q-TOF-MS, quadrupole time-of-flight mass spectroscopy; RP, reverse phase.

INTRODUCTION

Dandelion (*Taraxacum officinale*) has been extensively used in folk medicine for its curative properties, particularly against inflammation, arthritis and liver disorders (Bisset *et al.,* 1994; Schütz *et al.,* 2006). In more recent times, various parts of dandelion have been investigated for their antioxidant (Hu and Kitts, 2005; Park *et al.,* 2011), anti-inflammatory (Kashiwada *et al.,* 2001; Jeon *et al.,* 2008), antidiabetic (Hussain *et al.,* 2004; Petlevski *et al.,* 2003) and anticancer (Koo *et al.,* 2004; Sigstedt *et al.,* 2008) properties. Extracts of medicinal herbs and spices are generally considered to be rich sources of antimicrobial compounds (Cowan, 1999), however, only a small number of studies into the antimicrobial efficacy of dandelion have been undertaken and, in many cases, the biologically active compounds have not been identified. For example, a study by Sengul *et al.* (2009) investigated the antimicrobial properties of water and methanol extracts from aerial parts of dandelion against a number of microorganisms. Whilst the authors hypothesised that phenolic compounds were responsible for the observed activity, no compounds were identified. Contradictory to the study by Sengul *et al.* (2009), López-García *et al.* (2013) have since reported the inactivity of a similar extract against strains of *Escherichia coli* and *Staphylococcus aureus*, while also identifying several phenolic compounds including gallic acid, rutin, resveratrol, vanillic acid and sinapic acid. Although there appears to be some evidence for the antimicrobial efficacy of dandelion, there is a lack of clarity regarding the potency of this activity and indeed the compounds responsible.

In the present study, solid–liquid and dialysed extracts were prepared, and extracts possessing strong antimicrobial activity were further fractionated using normal phase (NP) flash chromatography. Subsequent structural elucidation of the active fractions was carried out using

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liquid chromatography solid phase extraction nuclear magnetic resonance (LC-SPE-NMR), while the accurate mass measurement was confirmed using LC mass spectroscopy (MS)/MS.

MATERIALS AND METHODS

Reagents and materials. Mueller-Hinton agar (MHB), BioDesignDialysis Tubing™ , with 3.5-kDa molecular weight cut-off (MWCO) and the HPLC-grade solvents *n*-hexane, diethyl ether, dichloromethane (DCM), ethyl acetate, methanol, acetonitrile, dimethyl sul phoxide (DMSO) deuterated acetonitrile (CD_3CN), water and formic acid were purchased from Fisher Scientific, Ltd (Dublin, Ireland). Magnesium sulphate ($MgSO_4$), iodonitrotetrazolium chloride (INT) and leucine enkephalin were obtained from Sigma-Aldrich, Ltd (Wicklow, Ireland) Spectra/Por® Biotech cellulose ester dialysis tubing with 100-kDa cut-off was acquired from Apex Scientific (Kildare, Ireland).

Plant material. Dandelion roots were purchased from Irish Organic Herbs, Ltd (Drumshanbo, Co., Leitrim, Ireland). A voucher specimen (DBN 27:2013) of dandelion was deposited in the Herbarium of the National Botanic Gardens (Dublin, Ireland). The species of dandelion was subsequently identified as *Taraxacum officinale* F.H. Wigg. The remaining roots were sliced (<10 mm) and freeze-dried (A12/60 Freeze Dryer, Frozen In Time, Ltd, York, England). The freeze-dried roots were blended to a fine powder using a Waring Commercial Blender (Christison Particle Technologies, Gateshead, UK) and stored at −80°C until further use.

Crude extraction and dialysis. Dandelion root powder (700 g) was extracted exhaustively at room temperature using a sequential solid–liquid extraction process with hexane, DCM, methanol and water in that order. The extraction process involved mixing powder and solvent (1:10) vigorously using a MaxQ6000 shaker (Thermo Scientific, IA, USA) for 3 h at 70 rpm. A Buchner funnel was used to filter the extract. The powder was washed three times using the same solvent followed by another 3 h extraction, again using the same solvent (1:10). After 3 h, the extract was filtered and washed as previously mentioned. Finally, the powder was extracted overnight using the same solvent (1:20). After filtering and washing, the powder was dried under nitrogen to remove residual solvent. Then, the powder was extracted by the next solvent in the sequential extraction process. Extracts of the same solvent were pooled together and dried using either a rotary evaporator (Rotavapor-R220, Bucchi, Ltd, Switzerland) at 40°C or a freeze-dryer and stored at −80°C. Solvent partitioning of the methanol extract was carried out by first dissolving the extract in water and then washing repeatedly with ethyl acetate (methanol–hydrophobic extract) in a separating funnel. The methanol hydrophobic partition was filtered through magnesium sulphate ($MgSO_4$), under suction, to remove traces of water. The extract was then dried at 40°C using a rotary evaporator. The remaining water-soluble extract (methanol–hydrophilic extract) was freeze-dried and reconstituted in a small volume of water. Fractionation of this extract based on molecular weight was performed using dialysis tubing with a MWCO of 3.5 kDa. The water crude extract was also subjected to fractionation by dialysis using tubing of 3.5 and 100 kDa MWCO respectively. All dialysis extracts were freeze-dried and stored at −80°C. In total, 11 extracts were generated from dandelion root (DR), which included hexane (E1), DCM (E2), ethyl acetate (E3), methanol–hydrophilic extract (E4), methanol–hydrophilic extract <3.5 kDa (E4 <3.5 kDa), methanol–hydrophilic extract >3.5 kDa (E4 >3.5 kDa), water (E5), water <3.5 kDa (E5 <3.5 kDa), water >3.5 kDa (E5 >3.5 kDa), water <100 kDa (E5 <100 kDa) and water >100 kDa (E5 >100 kDa) crude and dialysed extracts.

Bacterial strains and culture conditions. Extracts from each plant were tested for antimicrobial activity against the following strains of bacteria: *S. aureus* NCTC 8178, methicillin-resistant *S. aureus* (MRSA) clinical isolate (Causeway Hospital, Coleraine, Ireland), *Bacillus cereus* NCTC 7464, *E. coli* DSM 1103 and *Salmonella typhimurium* Salmonella Reference Collection B 69. The strains were stored on ceramic beads in glycerol at −80°C prior to use. A bead of each strain was streaked to a nutrient agar plate and incubated for 18 h at 37°C. A single colony was removed from each plate and inoculated into tubes containing 25 mL of sterile MHB and incubated for 22 h at 37°C. Overnight cultures were vortexed and aliquots diluted appropriately in sterile MHB to produce solutions containing log 6.0 ± 0.5 cells/mL. Cell numbers were confirmed by plate counting.

Minimum inhibitory concentration (MIC) antimicrobial assay. The MIC of each plant extract was carried out using a previously described microtitre method developed by Smyth *et al.* (2009). Each plant extract (2 mg/mL) was prepared by dissolving the material in sterile water (4% DMSO). In lane 1 of a 96-well plate, the following blanks and controls were added in triplicate: 100 µL of MHB (blank), 50 µL of gentamicin (0.2 mg/mL; negative control), 100 µL of bacteria (blank) and 50 µL sterile water (4% DMSO; positive control). A volume of 50 µL sterile water was added to wells 3–12 of lanes 2–8. Next, 100 µL of each sample was added to well 1 of lanes 2–8, while 75 µL of sample and 25 µL of sterile water were added to well 2 of the same lanes. Using a multichannel pipette, a serial dilution was carried out between well 1, 3, 5, 7, 9 and 11 of lanes 2–8, where the final volume of each well was 50 µL. The same procedure was carried out for wells 2, 4, 6, 8, 10 and 12 of lanes 2–8. All wells on the plate, with the exception of wells 1–3 and wells 7–9 in lane 1, were inoculated with 50 µL of bacteria, whereby the final volume of each well was 100 µL. This procedure was carried out for all bacterial strains. The plates were incubated at 37°C for 24 h using a Stuart SI50 orbital incubator (Rhys International, Ltd, Bolton, UK) at 30 rpm. After incubation, 40 µL of INT was added to each well using a multichannel pipette. Plates were further incubated for 1 h. The MIC of

each sample against a bacterial strain was determined as the lowest sample concentration at which no pink colour appeared (Eloff, 1998). This process was repeated in triplicate on separate days for each bacterial strain.

Flash chromatography. Fractionation of DRE3 (16 g) was carried out on a Varian IntelliFlash 310 Flash Chromatography (Analogix Semiconductor, Inc., CA, USA) system using an Agilent SuperFlash™ SF40-240g NP silica column (40.6 mm × 37.9 cm, 50 μm). Fractions were eluted with *n*-hexane–ethyl acetate mixtures, starting at 0% ethyl acetate (up to 100% ethyl acetate), in stepwise 10% increments of ethyl acetate. Further fractions were eluted with ethyl acetate–methanol mixtures, starting with 0% methanol (up to 100% methanol), in stepwise 20% increments of methanol. A total of 16 NP fractions (NPF1–16) were collected and dried at 40°C using a Laborata 4000 Efficient rotary evaporator. NPF4 (0.546 g) was fractionated further using an Agilent SuperFlash™ SF15-24g NP silica column (20.8 mm × 17.4 cm, 50 μm). Fractions were eluted with *n*-hexane–diethyl ether mixtures, starting with 0% diethyl ether, (up to 70% diethyl ether) in stepwise 10% increments. A final ramp to 100% diethyl ether was also carried out. A total of nine NPF4 fractions (NPF401–409) were collected and dried at 40°C using a rotary evaporator.

Liquid chromatography solid phase extraction nuclear magnetic resonance. The separation and isolation of NPF403, NPF406 and NPF5 was achieved on a system consisting of an Agilent 1200 HPLC (Agilent Technologies, GmBH, Germany) fitted with a Bruker diode array detector (DAD) detector (Bruker UK, Ltd, Coventry, UK), Spark Prospekt 2 system (Spark Holland BV, Emmen, Holland) and Gilson liquid handler (Gilson, Inc., Middleton, WI, USA). HySphere™ polydivinylbenzene-resin-filled SPE cartridges (10 × 2 mm, 10–12 μm; Spark Holland BV, Emmen, Holland) were used to trap resolved peaks from fractions. Samples were dissolved in methanol before centrifuging at 14,000 rpm. A binary solvent system of water (mobile phase A) and acetonitrile (mobile phase B) was used for the separation of each fraction using reverse phase (RP) columns. In each case, the gradients were optimised to achieve the best possible peak resolution. The optimised conditions are outlined in the succeeding texts.

NPF403. Separation was carried out using an Agilent Zorbax SB-C_8 (4.6 × 150 mm, 5 μm) RP column. The starting condition was 98:2 (A:B) with a ramp to 35:65 (A:B) by 15 min. A further gradient increase to 2:98 was completed by 44 min and held for 2 min. The column was returned to starting conditions of 98:2 (A:B) by 50 min. The column oven temperature was set at 40°C, and the wavelength of the DAD was recorded at 265, 216 and 205 nm.

NPF406. The separation of NPF406 was achieved on an Agilent Zorbax SB-C_8 (4.6 × 150 mm, 5 μm) RP column under isocratic conditions of 50:50 (A:B). The duration of the run-time was 40 min. The column oven temperature was set at 40°C, and the wavelength of the DAD detector was recorded at 283 nm.

NPF5. Separation was achieved using an Agilent Zorbax SB-C_{18} (4.6 × 150 mm, 5 μm) RP column. The starting condition was 98:2 (A:B) followed by a gradient increase to 60:40 (A:B) by 32 min, with a ramp to 2:98 (A:B) by 36 min and held for 40 min. The column was finally reconditioned to 98:2 (A:B) by 45 min. The column oven temperature was set at 40°C, and the wavelength of the DAD detector was recorded at 323 nm.

NMR analysis. ^{1}H-NMR analysis was carried out on all trapped isolates from NPF403, NPF406 and NPF5. The spectra were referenced to the residual CD_3CN signal. Because of the presence of residual solvent, a 1D nuclear Overhauser effect spectroscopy using double pre-saturation during relaxation delay and mixing time was used to acquire the spectra. A total of 32-k data points were recorded over a sweep width of 10 000 Hz, with 512 scans. An exponential line broadening of 1 Hz was imposed on the accumulated data before Fourier transformation. Structures were confirmed using correlation spectroscopy (COSY) and total correlation spectroscopy sequences.

LC-MS/MS analysis. Liquid chromatography solid phase extraction nuclear magnetic resonance isolates were diluted 1 in 2 with acetonitrile and transferred from NMR tubes to 2 mL amber vials containing 200 μL glass inserts. Mobile phase A (water + 0.1% formic acid) and mobile phase B (acetonitrile + 0.1 % formic acid) were used for chromatographic separation. The starting condition of each run was 98:2 (A:B) and held for 2 min. By 14 min, the gradient had increased to 2:98 and was held for 5 min. Finally, the column was reconditioned to 98:2 (A:B) by 21 min and held for 4 min. The column oven temperature was set at 50°C. Quadrupole time-of-flight MS (Q-TOF MS) was conducted in negative mode electrospray ionisation (ESI). The source temperature was 120°C, and the desolvation temperature was 300°C. In each case, nitrogen was used as the desolvation gas (800 L/h) and cone gas (50 L/h). The cone voltage was 40 V, while the lock mass was set to leucine enkephalin $[M+H]^+$ (556.2771 *m*/*z*). Samples were analysed in negative mode in the range *m*/*z* 150–1200.

RESULTS AND DISCUSSION

Antimicrobial activity of crude and dialysed extracts

The antimicrobial efficacy of crude and dialysed extracts from dandelion root were tested against three Gram positive (*S. aureus*, MRSA and *B. cereus*) and two Gram negative (*E. coli* and *S. typhimurium*) bacterial strains. The hexane crude extract (DRE1) demonstrated antimicrobial activity against *B. cereus* (MIC = 1000 μg/mL), while the DCM extract (DRE2) was active against *S. aureus* and MRSA (MICs = 1000 μg/mL). DRE3 showed the strongest antimicrobial activity, in relation to crude dandelion extracts, and was active against all

Table 1. Antimicrobial activities of normal phase (NP) flash chromatography fractions of DRE3, represented as minimum inhibitory concentrations (MICs), against selected strains of *S. aureus*, MRSA and *B. cereus*

		MIC (μg/mL)		
Extract	Yield (g)	*S. aureus* NCTC 8178	MRSA clinical	*B. cereus* NCTC 7464
Crude	—	500	500	250
NPF1	0.124	—	—	—
NPF2	0.488	—	—	—
NPF3	0.416	250	250	—
NPF4	0.546	93.75	62.5	93.75
NPF5	0.314	125	187.5	187.5
NPF6	0.336	—	—	375
NPF7	0.221	250	250	375
NPF8	0.249	250	250	375
NPF9	0.805	—	500	—
NPF10	1.672	250	187.5	187.5
NPF11	1.259	—	—	—
NPF12	5.210	—	—	—
NPF13	1.520	—	—	—
NPF14	0.985	—	—	—
NPF15	0.384	—	—	—
NPF16	2.024	—	—	—

Values are means of replicate assays (n = 3).

three Gram positive strains at an MIC = 500 μg/mL. However, neither DRE3 nor any other crude or dialysed extract was active against *E. coli* and *S. typhimurium*. A study by Sengul *et al.* (2009) investigated the zonal inhibition of crude methanol and water Soxhlet extracts from the aerial parts of dandelion against 32 bacterial strains. The strongest activity was seen in the methanol extract, which was active (MICs = 300 μg/mL) against ten bacterial strains particularly against *B. cereus* (6 cm radius). Meanwhile, the water extract was only mildly active against *B. cereus* (1 cm radius). In addition, Sengul *et al.* (2009) attributed the antimicrobial activity of the methanol extract to the presence of phenolics and subsequently linked this to its antioxidant activity. However, the same study was unable to demonstrate any activity in either the methanol or water extract against *S. aureus*. A more recent study by López-García *et al.* (2013) reported that an aqueous methanol (90% v/v) extract of dandelion flowers demonstrated no inhibition of bacterial growth against *S. aureus* and *E. coli* despite identifying a number of phenolic compounds (i.e. gallic acid, rutin, resveratrol, vanillic acid and sinapic acid) in this extract. In the present study, DRE3 was derived from a methanol extract of dandelion root material and was active (MIC = 500 μg/mL) against *B. cereus* and *S. aureus*, although no inhibition of *E. coli* was observed. This suggests that dandelion root has greater antimicrobial efficacy against *S. aureus* than aerial parts of dandelion. Based on the activity exhibited by DRE3, further fractionation of this crude extract was carried out using NP flash chromatography.

Antimicrobial activity of NP flash fractions

Fractionation of DRE3 was carried out in order to further investigate the antimicrobial efficacy of dandelion root. NP fractionation by flash chromatography led to the generation of 15 fractions derived from various solvent mixtures of *n*-hexane–ethyl acetate (NPF1–11) and ethyl acetate–methanol (NPF12–16). The antimicrobial activities of these fractions are outlined in Table 1. As was the case with the DRE3 crude extract, the subsequent NP fractions of DRE3 were unable to inhibit the growth of *E. coli* and *S. typhimurium* strains. Therefore, results for these bacteria have been omitted from Table 1. In relation to the Gram positive strains, seven NP fractions demonstrated antimicrobial activity at varying concentrations (MICs = 62.5–500 μg/mL). NPF4 (*n*-hexane–ethyl acetate, 70:30) showed the strongest inhibition against *S. aureus* (MIC = 93.75 μg/mL), MRSA (MIC = 62.5 μg/mL) and *B. cereus* (MIC = 93.75 μg/mL), while NPF5 (*n*-hexane–ethyl acetate, 60:40) also demonstrated a significant increase in activity (MICs = 125–187.5 μg/mL) against these strains in comparison with the DRE3 crude extract. Preliminary HPLC analysis was carried out on NPF4 and NPF5 to determine their complexity and suitability for further compound characterisation. Based on this analysis, NPF5 was suitable for characterisation at this stage, while NPF4 was too complex (data not shown) and required additional fractionation prior to chemical characterisation. Further NP fractionation by flash chromatography of NPF4 was carried out with solvent mixtures of *n*-hexane–diethyl ether. From the nine fractions generated, seven of these were active at varying concentrations (MICs = 46.88–375 μg/mL) against the Gram negative strains (Table 2). The strongest activities were observed in NPF403 (*n*-hexane–diethyl ether, 80:20; MICs = 46.88–93.75 μg/mL) and NPF406 (*n*-hexane–diethyl ether, 50:50; MICs = 62.5–93.75 μg/mL) respectively. As a result, both these fractions and NPF5 were selected for further compound isolation and characterisation using hyphenated techniques.

NMR and LC-MS/MS analysis

Separation methods for NPF403, NPF406 and NPF5 were developed using HPLC. A total of two isolates

Table 2. Antimicrobial activities of normal phase (NP) flash chromatography fractions of NPF4, represented as minimum inhibitory concentrations (MICs), against selected strains of *S. aureus*, MRSA and *B. cereus*

Extract	Yield (mg)	MIC (μg/mL)		
		S. aureus NCTC 8178	MRSA clinical	*B. cereus* NCTC 7464
Crude (NPF4)	—	93.75	62.5	93.75
NPF401	4.7	—	—	—
NPF402	5.4	—	—	—
NPF403	63.7	46.88	93.75	93.75
NPF404	89.8	375	375	250
NPF405	279.0	375	375	375
NPF406	15.7	62.5	93.75	62.5
NPF407	12.7	375	375	250
NPF408	12.4	187.5	187.5	250
NPF409	12.1	93.5	187.5	93.5

Values are means of replicate assays (n = 3).

(compounds **1** and **2**) from NPF406 and three isolates (compounds **3–5**) from NPF5, the major compounds in these fractions, were trapped separately on SPE cartridges for further analysis by NMR. Although a range of column chemistry such as RP (C_{18} and C_8), hydrophilic interaction liquid chromatography phase, NP and mixed cyano phase were used to try and resolve analytes within NPF403, sufficient separation could not be achieved.

Compounds **1** and **2** (Fig. 1) gave signals indicative of unsaturated fatty acids, with a peak in each spectrum corresponding to a terminal methyl group at 0.97 and 0.98 ppm respectively. Further peaks were observed for saturated CH_2 bonds from 2.04 to 2.00 ppm and unsaturated CH bonds between 6.58 and 5.50 ppm. The spectra of both compounds also showed a peak at approximately 4.20 ppm because of the presence of a CH adjacent to an alcohol group in a fatty acid chain. In the case of compound **1**, a significant amount of impurities was noted, which complicated the analysis. However, there were six hydrogen atoms identified as unsaturated corresponding to a triene, with two of the double bonds being separated from the final double bond by a single unsaturated carbon, which can be seen in the triplet at 3.02 ppm. In the case of compound **2**, the saturated portion and alcohol signal were similar to compound **1**, with significant differences noted in the unsaturated portion. There were four hydrogen signals in this region arising from two double bonds in conjugation. Because of a splitting value of 16 Hz between CH10 at 5.73 ppm and CH11 at 6.57 ppm with CH-10 and CH-11 separated by a double bond and 11 Hz between C11 and C12 at 6.07 ppm with CH-11 and CH-12 separated by a single bond, these were determined as a *trans* bond between C10 and C11 and a *cis* bond between C12 and C13 at 5.73, whereby the proton giving rise to the signal at 4.2 ppm was adjacent to C10, therefore at C9. These results were confirmed by COSY and by comparison with compounds reported in the literature (Kuhn *et al.,* 1989; McRae *et al.,* 2008). Subsequently, these compounds were identified as the hydroxylated fatty acids: 9-hydroxyoctadecatrienoic acid (9-HOTE) and 9-hydroxyoctadecadienoic acid (9-HODE, α-dimorphecolic acid). The accurate mass measurement of compound **1** using LC-MS/MS showed an elemental composition of $C_{18}H_{29}O_3$ ($[M-H]^-$ observed m/z 293.2115, calculated m/z 293.2117), while the elemental composition of compound **2** was that of $C_{18}H_{31}O_3$ ($[M-H]^-$ observed m/z 295.2270, calculated m/z 295.2273). McRae *et al*. (2008) also highlighted the antimicrobial activity of both 9-HOTE and 9-HODE, which were isolated from a methanol extract of *Planchonia careya*. These authors reported that HOTE demonstrated antimicrobial activity against MRSA and *S. aureus* (MICs = 9.0 mg/mL), while this compound was less active against vancomycin-resistant *Enterococcus* and *E. coli* (MICs ≥36.0 mg/mL). 9-HODE exhibited stronger antimicrobial activities against the same compounds in each case (MICs = 0.5–16 mg/mL). However, both compounds were unable to inhibit the growth of *B. cereus*. In contrast, the present study has demonstrated the antimicrobial activity of a fraction (NPF4) from dandelion, containing HOTE and HODE, against *B. cereus* (MIC = 62.5 μg/mL) as well as lower MIC values against MRSA and *S. aureus* (MICs = 62.5 and 93.75 μg/mL) respectively. The variability in MIC values between the present study and that of McRae *et al*. (2008) may be explained by the differences in bacterial cell numbers prior to inoculation. In the present study, the cell numbers were log 6.0 ± 0.5 cells/mL, whereas McRae *et al*. (2008) used higher cell concentrations approximately log 8.2 cells/mL.

Meanwhile, NMR analysis identified compound **3** as the compound vanillin (4-hydroxy-3-methoxybenzaldehyde, Fig. 1). This compound gave a very clear methoxy signal at 4.02 ppm and a peak corresponding to an aromatic aldehyde at 9.89 ppm. The remaining signals in the spectra corresponded to aromatic multiplets from two protons at

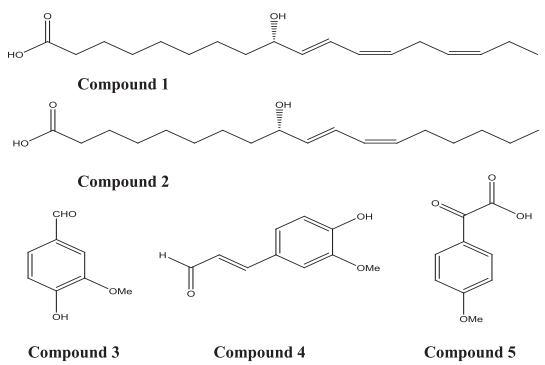

Figure 1. Structures of compounds **1–6** characterised from NPF406 and NPF5 using NMR and LC-MS/MS.

7.53 ppm and a doublet arising from a single proton at 7.08 ppm. The doublet showed a splitting pattern of 8.9 Hz, suggesting a proton with the remaining protons *ortho* and *para* to it. The splitting values and the positions of these peaks allowed the substitution pattern on the ring to be determined and compared with both calculated and known values (Furniss *et al.,* 1989). Accurate mass measurement confirmed the identity of compound **3** as vanillin, where the elemental composition was that of $C_8H_7O_3$ ($[M-H]^-$ observed *m/z* 151.0394, calculated *m/z* 151.0395). A study by Cerrutti *et al.* (1997) described the antimicrobial activity of vanillin as an additive in strawberry puree to prolong its shelf life. At a concentration of 3 mg/mL, vanillin was able to prevent the growth and detection of both *Bacillus coagulans* and *Lactobacillus delbrueckii* over 60-day storage. A study by Katayama and Nagai (1960) reported that vanillin prevented the growth of *Bacillus subtilis*, *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella enteriditis*.

Compound **4** was identified as coniferyl aldehyde ((*E*) 4-hydroxy-3-methoxyphenyl-prop-2-enal, Fig. 1), whereby ^{1}H-NMR analysis showed one peak at 4.00 ppm, indicative of a methoxy substituent on an aromatic ring. The aromatic peaks could be seen at 6.98, 7.35 and 7.26 ppm respectively. The presence of a double doublet at 6.73 ppm, with a splitting of $J = 16$ Hz and $J = 8.4$ Hz, and a doublet at 7.60 ppm, with splitting of $J = 16$ Hz, corresponded to a *trans* double bond adjacent to the aromatic structure. A doublet at 9.70 ppm had a splitting of $J = 8.4$ Hz because of the presence of an aldehyde adjacent to the double bond. Additional peaks were observed because of the presence of an impurity tentatively identified as a coumaric acid derivative, which gave rise to peaks at 7.18, 6.85 and 3.74 ppm. LC-MS/MS analysis of compound **4** confirmed the identification of coniferaldehyde, which had an elemental composition of $C_{10}H_{10}O_3$ ($[M-H]^-$ observed *m/z* 177.0544, calculated *m/z* 177.0552). Coniferaldehyde has previously been isolated (80% aqueous methanol extract) from the culms of *Phyllostachys bambusoides* (Kim *et al.,* 2011). The same study also investigated the antimicrobial activity of this compound against strains of *Streptococcus mutans* (MIC = 300 μg/mL) and *Streptococcus sobrinus* (MIC = 567 μg/mL).

In relation to compound **5**, the ^{1}H NMR spectra revealed a *para* di-substituted aromatic compound with distinctive doublets at 7.97 and 7.05 ppm. Similar to compound **4**, a methoxy substituent of the aromatic ring was seen at 4.01 ppm. Accurate mass measurement of compound **5** determined the molecular formula to be that of $C_9H_8O_4$ ($[M-H]^-$ observed *m/z* 179.0349, calculated *m/z* 179.0344), which suggests that the only possible structure for this compound is the keto acid shown in Fig. 1 (*p*-methoxyphenylglyoxylic acid). However, no antimicrobial activity for this compound has previously been reported.

Compound **1**: UV (MeCN:H_2O): λ_{max} 280 nm. ^{1}H NMR spectral data (500 MHz, CD_3CN) δH 6.58, 1H, m, (C11H), 6.08, 1H, m (C12H), 5.78–5.43, 1H, m, (C10H), 5.57–5.43, 3H, bm, (C13, 15, 16H), 4.20, 1H, m, (C9H), 3.02, 2H, t, $J = 7.3$ Hz (C14H), 2.04–2.00, bm, (CH2), 0.97, 3H, t, $J = 7.1$ Hz, (C18H). ESI-Q-TOF-MS observed *m/z* 293.2115 $[M-H]^-$ (calculated *m/z* 293.2117, $C_{18}H_{29}O_3$).

Compound 2: UV (MeCN:H_2O): λ_{max} 279 nm. ^{1}H NMR spectral data (500 MHz, CD_3CN) δH, 6.57, 1H, dd, J = 16, 11 Hz, (C11H), 6.07, 1H t, J = 11 Hz, (C12H), 5.73, 1H dd, J = 16, 6.4 Hz, (C10H), 5.51, 1H, m, (C13H), 4.14, 1H, t, J = 5.8 Hz(C9H), 1,64, 3H, t, J = 7.2 Hz (C14H), 1.53, 2H, m, (C8H), 1.49–1.40, bm, (CH2 × 9), 0.98, 3H, t, J = 7.5 Hz, (C18H). ESI-Q-TOF-MS observed m/z 295.2270 $[M-H]^-$ (calculated m/z 295.2273, $C_{18}H_{31}O_3$).

Compound 3: UV (MeCN:H_2O): λ_{max} 203, 230, 279 and 310 nm. ^{1}H NMR spectral data (500 MHz, CD_3CN) δH, 9.89, 1H, s (CHO), 7.53–7.51, 2H, m (C2H, C6H), 7.08, 1H, d, J = 8.9 Hz, (C5H), 4.02, 3H, s, (OMe). ESI-Q-TOF-MS observed m/z 151.0394 $[M-H]^-$ (calculated m/z 151.0395, $C_8H_7O_3$).

Compound 4:UV (MeCN:H_2O): λ_{max} 340 nm. ^{1}H NMR spectral data (500 MHz, CD_3CN) δH 9.7, 1H, d, J = 7.8 Hz (CHO), 7.60, 1H, d, J = 16 Hz, (CH1′), 7.35, 1H, d, J = 1.6 Hz, (C2H), 7.26, 1H, dd, J = 1.6, 8.2 Hz (C6H), 6.98, 1H d, J = 8.2 Hz, (C5H), 6.73, 1H, dd, J = 16, 7.8 Hz (CH2′), 4.00, 3H, s, (OMe). ESI-Q-TOF-MS observed m/z 177.0544 $[M-H]^-$ (calculated m/z 177.0552, $C_{10}H_9O_3$).

Compound 5:UV (MeCN:H_2O): λ_{max} 299 nm. ^{1}H NMR spectral data (500 MHz, CD_3CN) δH, 7.97, 2H, d, J = 8.7 Hz (C2, C6H), 7.05, 2H, d, J = 8.7 Hz (C3, C5H), 4.01, 3H, s, (OMe). ESI-Q-TOF-MS observed m/z 179.0349 $[M-H]^-$ (calculated m/z 179.0344, $C_9H_8O_4$).

CONCLUSION

The antimicrobial efficacy of crude and dialysed extracts from freeze-dried dandelion root was measured against a range of common food borne pathogens and MRSA. The methanol hydrophobic crude extract (DRE3) possessed the strongest antimicrobial activity against *S. aureus*, MRSA and *B. cereus* strains (250–500 μg/mL), while no activity was observed against *E. coli* and *S. typhimurium*. Fractionation of DRE3 using NP flash chromatography led to the generation of 16 fractions of which NPF4 (MICs = 62.5–93.75 μg/mL) and NPF5 (MICs = 125–187.5 μg/mL) exhibited the strongest inhibition of growth against Gram negative bacterial strains. Further NP fractionation of NPF4 resulted in two fractionates (NPF403 and NPF406) with notably increased antimicrobial activity (MICs = 46.88–187.5 μg/mL). Despite using a number of diverse chromatographic columns to resolve the analytes present in NPF403, a suitable separation could not be achieved. Meanwhile, the isolation and characterisation of two hydroxyl fatty acids (NPF406) and three phenolic based compounds (NPF5) were carried out using LC SPE NMR, while the molecular mass of each compound was confirmed by LC-MS/MS. As these compounds formed the major constituents of fractions NPF406 and NPF5, it is likely that they are responsible for their antimicrobial activity

against the selected Gram positive bacteria, further strengthening the use of dandelion root as a source of natural antimicrobial compounds.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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