Pharmacognosy Communications
Volume 5 | Issue 2 | Apr-Jun 2015
www.phcogcommn.org

Research Article

Inhibitory activity of Australian culinary herb extracts against the bacterial triggers of selected autoimmune diseases

Anton Maen[1], Ian Edwin Cock,[1,2*]

[1]*School of Natural Sciences, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland 4111, Australia*
[2]*Environmental Futures Research Institute, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland 4111, Australia*

ABSTRACT

Introduction: High antioxidant capacities have been linked to the treatment of rheumatic diseases and in the inhibition of microbial growth. Recent reports have identified several native Australian culinary herbs with high antioxidant capacities. Despite this, several of these species are yet to be tested for the ability to inhibit the growth of the bacterial triggers of autoimmune inflammatory diseases. **Methods:** Solvent extracts prepared from selected Australian culinary herbs were analysed for antioxidant capacity by the DPPH free radical scavenging assay. Growth inhibitory activities against bacterial species associated with initiating rheumatoid arthritis and ankylosing spondylitis were determined by disc diffusion assay and quantified by MIC determination. Toxicity was determined by *Artemia franciscana* bioassay. **Results:** Methanolic extracts of most plant species displayed relatively high antioxidant contents (equivalent to approximately >10 mg of vitamin C per gram of fruit extracted). Most aqueous extracts also contained relatively high antioxidant capacities. The ethyl acetate and chloroform extracts generally had lower antioxidant capacities than the more polar extracts. In contrast, the hexane extracts of all species had low antioxidant contents (generally < 0.5 mg of vitamin C per gram of fruit extracted). Interestingly, the bacterial growth inhibitory activity of the extracts did not correlate with their antioxidant capacities. With the exception of native thyme, the herb extracts with the highest antioxidant capacities had only low antibacterial activity, with MIC values generally well in excess of 4000 μg/ml against all bacterial species. In contrast, many of the ethyl acetate extract and hexane extracts, which had low range antioxidant capacities (generally < 5 mg ascorbic acid equivalents/g extracted), had potent bacterial growth inhibitory activity with many MIC values substantially below 1000 μg/ml). The river mint ethyl acetate extract displayed toxicity in the *Artemia franciscana* bioassay (LC50 = 186 μg/mL). All other extracts were nontoxic. **Conclusion:** The lack of toxicity and inhibitory activity against microbial triggers of rheumatoid arthritis and ankylosing spondylitis by the Australian native culinary herb extracts indicates their potential in the treatment and prevention of these diseases.

Key words: Ankylosing spondylitis, Functional foods, Native thyme, Native sage, Native basil, River mint, Rheumatoid arthritis, Wattle seed.

INTRODUCTION

Herbs and spices are useful to provide flavouring, aroma and visual characteristics to food. In addition, many edible botanical products have been used as traditional medicines by multiple cultures. Indeed, the highly spiced nature of Indian cuisine and Ayurveda are intertwined.[1] Ayurvedic practitioners focus on disease prevention and holistic health promotion, with an emphasis on the diet. A large number of herbs and spices are common between Ayurveda and Indian cooking. For example, turmeric (a curry component) is used in Ayurveda to treat jaundice; basil is used as a cardio-protectant, cinnamon for circu-

*Correspondence autor:
Dr. I. E. Cock
School of Natural Sciences Environmental Futures Research Institute, Nathan Campus, Griffith University,
170 Kessels Rd, Nathan, Queensland 4111, Australia
E-mail: I.Cock@griffith.edu.au
DOI: 10.5530/pc.2015.2.4

latory disorders, mace for gastric infections and ginger as a universal medicine for a wide variety of complaints. Similarly, herbs and spices used in other Asian, European and African cuisines as well as food from the Americas, have also been used medicinally. Scientific investigation has verified the therapeutic potential of many of these. Curcumin has been identified as an anticancer component of turmeric.[2] Garlic, green tea, rosemary, sage thyme and saffron have been linked with a reduction of blood cholesterol, immunomodulatory and anticancer activity.[3]

Despite several notable exceptions, much less is known about the use of Australian culinary herbs and spices as therapeutic agents. *Tasmannia lanceolata* (Tasmanian pepper) is used as both an herb (leaves) and as a spice (berry) in the cooking of both Australian Aborigines and by European settlers.[4,5] Australian Aborigines also used *T. lanceolata* as a therapeutic agent to treat stomach disorders and as an emetic, as well as general usage as a tonic.[5] Reports also exist of the use of *T. lanceolata* by Australian Aborigines for the treatment and cure of skin disorders, venereal diseases, colic, stomach ache and as a quinine substitute.[5] Later, European colonists also recognized the therapeutic potential of *T. lanceolata* and the bark was used as a common substitute for other herbal remedies (including those derived from the related South American Winteraceae species, *Drimys winteri* (winter bark)[6] to treat scurvy due to its high antioxidant content.[5,7]

Many other Australian plant species also have a history of usage as food flavourings and are considered culinary equivalents of herbs from other parts of the world for which therapeutic properties are well established. *Prostanthera rotundifolia* (native thyme) may be used as a replacement for European thyme (*Thymus vulgaris*), *Salvia plebeian* (native sage) is a culinary equivalent of sage (*Salvia officinalis*), and *Mentha australis* (river mint) has similar taste and aroma characteristics to spearmint (*Mentha spicata*). All of these international herbs have been shown to have therapeutic properties including antimicrobial, anti-inflammatory and anticancer activities.[3] In contrast, there is limited documentation of the ethnopharmacological usage of the Australian herbs and an understanding of their medicinal properties is lacking. The current study was undertaken to test the ability of Australian culinary herbs to inhibit the growth of bacteria associated with autoimmune inflammatory diseases.

Autoimmune inflammatory disorders (e.g. rheumatoid arthritis, ankylosing spondylitis) are a group of debilitating conditions which afflict genetically susceptible individuals. There are no cures for any of these conditions. Instead, current treatment strategies aim to alleviate the symptoms (particularly pain, swelling and inflammation) with analgesics and anti-inflammatory agents and/or to modify the disease process through the use of disease modifying drugs. None of these treatments is ideal as prolonged usage of these drugs is often accompanied by unwanted side effects and toxicity.[8] There is a need to develop safer, more effective treatments for these conditions which will not only alleviate the symptoms, but may also cure or prevent the disease. A greater understanding of the onset and progression of these disorders should greatly assist in more relevant drug discovery and development.

The causes of the autoimmune inflammatory disorders are currently not well understood. However, it is generally accepted that they are immune disorders triggered in susceptible individuals by specific microbial infections. Recent serotyping studies have identified several of the bacterial triggers of some of these conditions and the bacterial antigens responsible for the induction of an immune response (Table 1). The major microbial trigger of rheumatoid arthritis has been identified as *Proteus mirabilis*,[9] a normal part of the human gastrointestinal flora. Similarly, *Klebsiella pneumoniae* has been shown to initiate

Table 1: The bacterial triggers of rheumatoid arthritis and ankylosing spondylitis as well as the bacterial antigen and host susceptibility antigen sequences.

Disease	Bacterial Trigger	Bacterial Antigen	Bacterial Sequence	Host Antigen	Host Sequence	References
Rheumatoid arthritis	*Proteus mirabilis* and possibly also other Proteus spp.	haemolysin	ESRRAL	MHC class 2 allele HLA-DR4	EQ/KRRAA	9-12
		urease	IRRET	type XI collagen	LRREI	11, 13
Ankylosing spondylitis	*Klebsiella pneumoniae*	nitrogenase reductase enzyme	QTDRED	MHC class 1 allele HLA-B27	QTDRED	10, 14
		pullulanase	DRDE	MHC class 1 allele HLA-B27	DRED	15
		pullulanase		types I, III and IV collagen	GxP	16

ankylosing spondylitis.[10] The development of antibiotic agents targeted at the specific bacterial triggers of autoimmune inflammatory disorders would enable afflicted individuals to target these microbes and thus prevent the onset of the disease and reduce the severity of the symptoms once the disease has progressed. Antimicrobial plant extracts with high antioxidant contents are particularly attractive as they may treat the symptoms of inflammation as well as blocking the microbial trigger and thus have pluripotent effects.

MATERIALS AND METHODS

Plant source and extraction

All plant material was obtained from Taste Australia Bush Food as verified dried herbs and spices. Voucher samples have been stored in the School of Natural Sciences, Griffith University. Individual 1 g quantities of the ground plant material were weighed into separate tubes and 50 ml of methanol, water, ethyl acetate, chloroform or hexane were added. All solvents were obtained from Ajax and were AR grade. The ground plant materials were individually extracted in each solvent for 24 hours at 4°C with gentle shaking. The extracts were subsequently filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant dry extract was weighed and redissolved in 10 ml deionised water.

Qualitative phytochemical studies

Phytochemical analysis of the culinary herb extracts for the presence of saponins, phenolic compounds, flavonoids, polysteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.[17-19]

Antioxidant capacity determination

The antioxidant capacity of each sample was assessed using the DPPH free radical scavenging method[19] with modifications. Briefly, DPPH solution was prepared fresh each day as a 400 µM solution by dissolving DPPH (Sigma) in AR grade methanol (Ajax, Australia). The initial absorbance of the DPPH solution was measured at 515 nm using a Molecular Devices, Spectra Max M3 plate reader and did not change significantly throughout the assay period. A 2 ml aliquot of each extract was evaporated and the residue resuspended in 2 ml of methanol. Each extract was added to a 96-well plate in amounts of 5, 10, 25, 50, 75 µl in triplicate. Methanol was added to each well to give a volume of 225 µl. A volume of 75 µl of the fresh DPPH solution was added to each well for a total reaction volume of 300 µl. A blank of each extract concentration, methanol solvent, and DPPH was also performed in triplicate. Ascorbic acid was prepared fresh and examined across the range 0-25 µg per well as a reference and the absorbances were recorded at 515. All tests were performed in triplicate and triplicate controls were included on each plate. The antioxidant capacity based on DPPH free radical scavenging ability was determined for each extract and expressed as µg ascorbic acid equivalents per gram of original plant material extracted.

Antibacterial screening

Test microorganisms

All media was supplied by Oxoid Ltd. Reference strains of *Klebsiella pneumoniae* (ATCC31488), *Proteus mirabilis* (ATCC21721) and *Proteus vulgaris* (ATCC21719) were purchased from American Tissue Culture Collection, USA. All other clinical microbial strains were obtained from the School of Natural Sciences teaching laboratory, Griffith University. All stock cultures were subcultured and maintained in nutrient broth at 4°C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified disc diffusion assay.[20-24] Briefly, 100 µl of the test bacteria were grown in 10 ml of fresh nutrient broth media until they reached a count of approximately 10^8 cells/ml. An amount of 100 µl of bacterial suspension was spread onto nutrient agar plates. The extracts were tested for antibacterial activity using 5 mm sterilised filter paper discs. Discs were impregnated with 10 µl of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4°C for 2 hours before incubation with the test microbial agents. Inoculated plates were incubated at 30°C for 24 hours, then the diameters of the inhibition zones were measured in millimetres. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in at least triplicate. Mean values (± SEM) are reported in this study. Standard discs of ampicillin (2 µg) were obtained from Oxoid Ltd. and served as positive controls for antibacterial activity. Filter discs impregnated with 10 µl of distilled water were used as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of the extracts were determined as previously described.[25,26] Briefly, the plant extracts were diluted in deionised water and tested across a range of concentrations. Discs were impregnated with 10 µl of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above and graphs of the zone of inhi-

Table 2: The mass of dried extracted plant material, the concentration after resuspension, qualitative phytochemical screenings and antioxidant contents of the cullinary herb extracts.

Plant Species	Extract	Mass of Dried Extract (mg)	Resuspended Extract Concentration (mg/ml)	Total Phenolics	Water Soluble	Water Insoluble	Cardiac Glycosides	Saponins	Triterpenes	Polysteroids	Alkaloids (Meyer test)	Alkaloids (Wagners test)	Flavanoids	Tannins	Free Anthraquinones	Combined Anthraquinones	Antioxidant capacity (ug AA equivalency)
Wattle seed	M	88	8.8	++	+++	++	-	++	++	-	-	++	+++	-	-	-	3.9
	W	120	12	++	++	+	++	-	++	-	-	-	+++	-	-	-	0.6
	E	51	5.1	-	-	-	-	++	-	-	+	+	-	-	-	-	1
	C	190	19	+	-	-	-	-	-	-	-	+	+	-	-	-	0.4
	H	70	7	-	-	-	-	++	-	-	-	-	-	-	-	-	BDT
Native Thyme	M	171	17.1	+++	+++	++	++	+++	++	-	-	++	+++	++	-	-	11.1
	W	52	5.2	+++	+++	+++	++	+++	++	-	-	++	+++	++	++	-	5.1
	E	19	1.9	++	+	+	-	-	++	-	-	+	++	++	-	-	4.2
	C	130	13	+	-	-	-	-	++	-	-	-	-	-	-	-	0.5
	H	10	1	-	-	-	+	++	-	-	-	-	-	-	-	-	BDT
Native Sage	M	109	10.9	+++	+++	+++	++	+++	++	-	-	+	++	++	-	-	12.3
	W	25	2.5	+++	+++	++	++	+++	++	-	-	-	+++	++	-	-	6.5
	E	25	2.5	++	+	+	++	-	++	-	-	++	++	++	-	-	6.3
	C	140	14	-	-	-	-	-	-	-	-	-	-	-	-	-	6.7
	H	30	3	-	-	-	-	++	-	-	-	-	-	-	-	-	0.3
Native Basil	M	192	19.2	+++	+++	+++	-	+++	++	-	-	++	+++	++	-	-	11.7
	W	108	10.8	+++	+	++	++	+++	++	-	-	+	++	+	-	-	6.8
	E	35	3.5	++	+	+	-	-	++	-	-	-	++	++	-	-	1.3
	C	260	26	+	-	-	-	-	-	-	-	-	+	-	-	-	3.3
	H	30	3	-	-	-	+	++	-	-	-	-	-	-	-	-	0.6
Rivermint	M	120	12	+++	+++	+++	++	+++	++	-	-	++	+++	++	+	-	10.6
	W	30	3	++	++	++	++	-	++	-	-	-	+++	-	-	-	5
	E	2	0.2	+	+	++	-	-	++	-	-	-	+	++	-	-	0.7
	C	140	14	+	-	-	-	-	-	-	-	-	+	-	-	-	1.1
	H	60	6	-	-	-	-	-	-	-	-	-	-	-	-	-	BDT

M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; +++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. AA = ascorbic acid. BDT = below detection threshold.

bition versus concentration were plotted for each extract. Linear regression was used to calculate the MIC values.

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/ml solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay.[27-29] Briefly, 400 µl of seawater containing approximately 48 (mean 47.8, n=132, SD 13.7) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 µl of diluted plant extracts or the reference toxin were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A negative control (400 µl seawater) was run in triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was observed within 10 seconds. After 24 h all nauplii were sacrificed and counted to determine the total

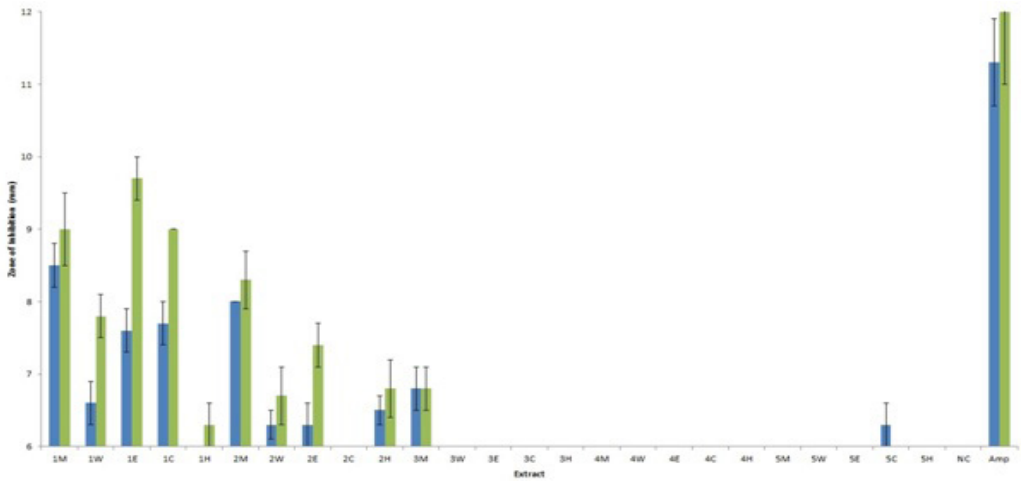

Figure 1: Antibacterial activity of the culinary herb extracts against *P. mirabilis* measured as zones of inhibition (mm). The blue bars represent the inhibitory activity against the reference strain (ATCC: 21721) and the green bars represent the zones of inhibition against the clinical strain. 1=wattle seed; 2=native thyme; 3=native sage; 4=native basil; 5=river mint; M=methanolic extract; W= water extract; E=ethyl acetate extract; C=chloroform extract; H=hexane extract; NC=0.5% DMSO; Amp=ampicillin (2 μg) control. Results are expressed as mean zones of inhibition ± SEM.

% mortality per well. The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.

Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments. One way ANOVA was used to calculate statistical significance between control and treated groups with a P value < 0.01 considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of the various dried Australian culinary herbs with the solvents yielded dried plant extracts ranging from 2 mg (river mint ethyl acetate extract) to 260 mg (native basil chloroform extract) (Table 2). Methanol gave relatively high yields of dried extracted material for all culinary herbs. Water and chloroform also generally gave high yields, whilst all other solvents extracted lower masses. The dried extracts were resuspended in 10 ml of deionised water, resulting in the extract concentrations shown in Table 2.

Qualitative phytochemical studies (Table 2) showed that methanol and water extracted the widest range and highest levels of phytochemicals. Both solvents extracted high levels of phenolics (both water soluble and insoluble phenolics) and flavonoids, as well as moderate to high levels of triterpenes and saponins. Interestingly, cardiac glycosides and alkaloids were also detected in low to moderate levels in most methanolic and aqueous extracts. With the exception of the wattle seed extracts, tannins were detected in the polar extracts of all culinary herbs. The ethyl acetate extracts generally extracted similar classes of phytochemicals to the methanolic and aqueous extracts, albeit at lower levels. In general, the levels of the screened phytochemical classes were below the level of detection in all chloroform and hexane extracts.

Antioxidant content

Antioxidant capacity (expressed as ascorbic acid equivalence) for the Australian culinary herbs are shown in Table 2. The antioxidant capacity ranged from below the level of detection to a high of 12.3 mg ascorbic acid equivalence per gram of dried plant material extracted (native sage methanolic extract). The methanol extracts of all herbs had higher antioxidant capacities than the corresponding water, ethyl acetate, chloroform or hexane extracts.

Antimicrobial activity

To determine the antimicrobial activity of the crude plant extracts, aliquots (10 μl) of each extract were tested in the disc diffusion assay. Both reference and clinical strains of *Proteus mirabilis* were inhibited by several of the Australian culinary herb extracts (Figure 1). The growth inhibition by the wattle seed extracts was particularly noteworthy, with much larger zones of inhibition recorded than for the other herb extracts. Indeed, with the exception of the hexane extract, all wattle extracts inhibited the growth of both *P. mirabilis* strains. The native thyme methanolic, aqueous and ethyl acetate extracts, and native sage methanolic extract also inhibited the growth of the reference and clinical strains of *P. mirabilis*. A greater susceptibility of the clinical strain than the reference strain was noted for the control antibiotic and all inhibitory extracts.

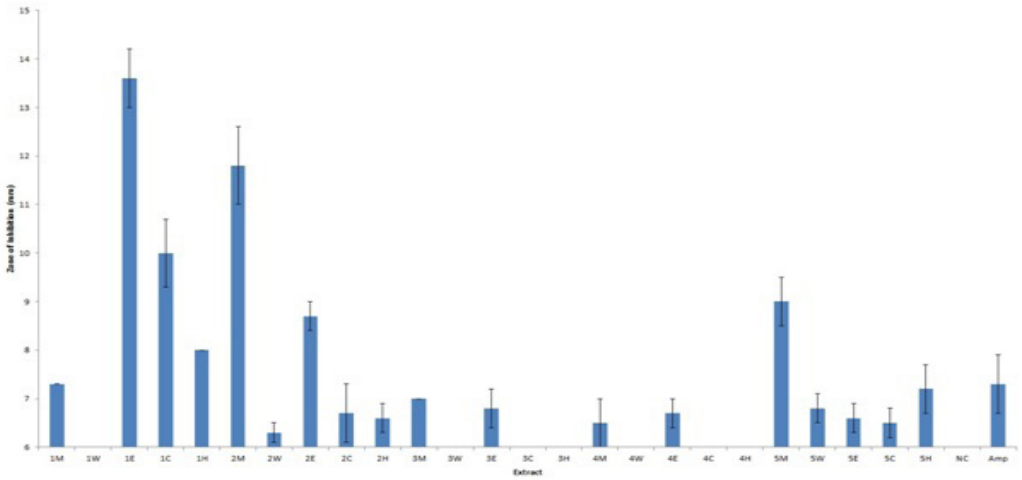

Figure 2: Antibacterial activity of the Australian culinary herb extracts against *P. vulgaris* (ATCC21719) measured as zones of inhibition (mm). 1=wattle seed; 2=native thyme; 3=native sage; 4=native basil; 5=river mint; M=methanolic extract; M=methanolic extract; W=water extract; E=ethyl acetate extract; C=chloroform extract; H=herb extracts; NC=0.5% DMSO; Amp=ampicillin (2 μg) control. Results are expressed as mean zones of inhibition ± SEM.

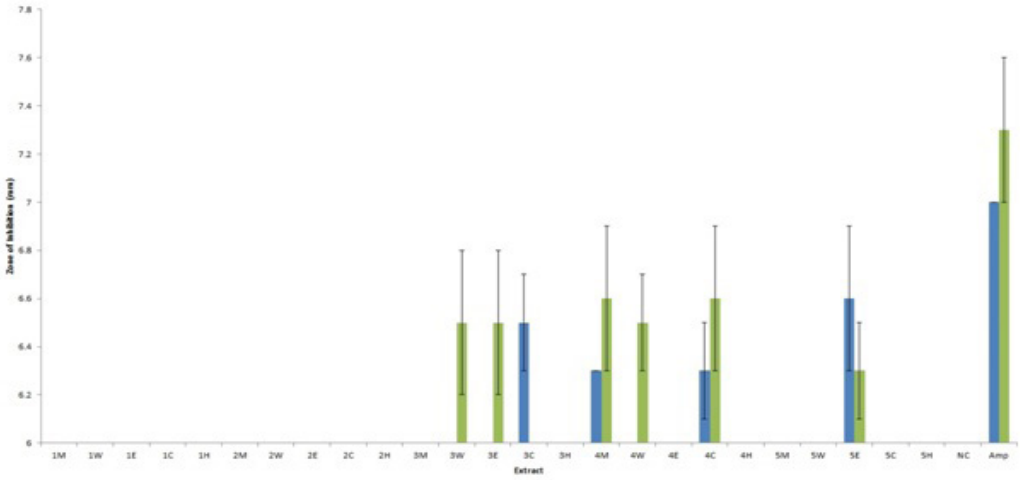

Figure 3: Antibacterial activity of the Australian culinary herb extracts against *K. pneumoniae* measured as zones of inhibition (mm). The blue bars represent the inhibitory activity against the reference strain (ATCC31488) and the green bars represent the zones of inhibition against the clinical strain. 1=wattle seed; 2=native thyme; 3=native sage; 4=native basil; 5=river mint; M=methanolic extract; M=methanolic extract; W=water extract; E=ethyl acetate extract; C=chloroform extract; H=hexane extract; NC=0.5% DMSO; Amp=ampicillin (2 μg) control. Results are expressed as mean zones of inhibition ± SEM.

The growth of *P. vulgaris* was substantially more susceptible to inhibition by the culinary herb extracts than were the *P. mirabilis* strains for most extracts. (Figure 2) The methanol and ethyl acetate extracts were generally more potent inhibitors of *P. vulgaris* growth than were the other extracts (as determined by the size of the zone of inhibition). The wattle seed ethyl acetate extract was particularly potent, with a zone of inhibition of approximately 13.5 mm. The wattle seed chloroform and native thyme methanolic extracts were also potent inhibitors of *P. vulgaris* growth, each with zones of inhibition ≥ 10 mm.

Both reference and clinical strains of *Klebsiella pneumoniae* were inhibited by several of the culinary herb extracts, albeit with relatively small zones of inhibition (Figure 3). Several native sage, native basil and river mint extractsinhibited one or both *K. pneumonia* strains. However, inhibition was weak (as judged by the zones of inhibition), with inhibition zones generally ≤ 6.6 mm in diameter. Notably, whilst the control antibiotic (2 μg ampicillin) did inhibit *K. pneumonia* growth, the inhibition zone was also relatively small (<7.5 mm) indicating that both the reference and clinical strains were ampicillin resistant.

The antimicrobial efficacy was further quantified by determining the MIC values for each extract against the microbial species which were determined to be susceptible. Several of the culinary herb extracts were effective

Table 3: Minimum inhibitory concentration (μg/ml) of the Australian culinary herb extracts.

Plant Species	Extract	*P. mirabilis* (reference strain)	*P. mirabilis* (clinical strain)	*P. vulgaris* (reference strain)	*K. pneumoniae* (reference strain)	*K. pneumoniae* (clinical strain)
Wattle seed	M	4886	4012	6151	-	-
	W	8335	7218	-	-	-
	E	1136	924	470	-	-
	C	1515	726	142	-	-
	H	-	6848	2630	-	-
Native Thyme	M	3104	1933	842	-	-
	W	4836	3995	4288	-	-
	E	1269	720	584	-	-
	C	-	-	>10000	-	-
	H	635	493	815	-	-
Native Sage	M	3748	6832	6220	-	-
	W	-	-	-	-	1660
	E	-	-	1326	-	1475
	C	-	-	-	7853	-
	H	-	-	-	-	-
Native Basil	M	-	-	>10000	>10000	>10000
	W	-	-	-	-	8453
	E	-	-	3118	-	-
	C	-	-	-	>10000	>10000
	H	-	-	-	-	-
Rivermint	M	-	-	4177	-	-
	W	-	-	2995	-	-
	E	-	-	220	196	218
	C	6774	-	>10000	-	-
	H	-	-	5525	-	-

Numbers indicate the mean MIC values of triplicate determinations. - indicates no inhibition. M=methanolic extract; W=aqueous extract; E=ethyl acetate extract; C=chloroform extract; H=hexane extract.

at inhibiting microbial growth (Table 3), with MIC values against many of the susceptible bacteria <1000 μg/ml (<10 μg impregnated in the disc), indicating the potential of these extracts in controlling multiple autoimmune inflammatory disorders. The MIC values determined for the native thyme hexane extract against the Proteus strains was particularly note worthy. This extract had MIC values in the range 500-800 μg/ml (5-8 μg impregnated in the disc) against all 3 Proteus species/strains. Therefore, native thyme may be useful as a functional food for people with rheumatoid arthritis. Similarly, the river mint ethyl acetate extract was a potent inhibitor of *K. pneumoniae* growth (both strains) with MIC values of approximately 200 μg/ml (2 μg impregnated in the disc). Therefore, river mint may be useful in controlling this condition by blocking its onset and lessening the symptoms. Several other of the Australian culinary herbs also demonstrated useful inhibitory activity, with MICs < 5000 μg/ml.

Quantification of toxicity

All extracts were initially screened undiluted in the assay (Figure 4). For comparison, the reference toxin potassium dichromate (1000 μg/ml) was also tested in the bioassay. The potassium dichromate reference toxin was rapid in its onset of mortality, inducing mortality within the first 3 hours of exposure and 100 % mortality was evident following 4-5 hours (results not shown). Similarly, most of the herb extracts displayed > 50 % mortality rates at 24 h and 48 h.

To further quantify the effect of toxin concentration on the induction of mortality, the extracts were serially diluted in artificial sea water to test across a range of concentrations in the Artemia nauplii bioassay at 24 hours. Table 4 shows the LC50 values of the culinary herb extracts towards *A. franciscana*. No LC50 values are reported for the wattle seed ethyl acetate and hexane extracts, the native thyme aqueous extract, as well as the native basil and river mint aqueous and hexane extracts as less than 50 % mortality was seen for all concentrations tested. All culinary herb extracts except river mint ethyl acetate extract were determined to be nontoxic, with LC50 values much greater than 1000 μg/ml following 24 h exposure.

DISCUSSION

Plant remedies are becoming increasingly sought after in the treatment of a myriad of diseases and disorders due both to their perception of greater safety than many synthetic drugs, and the failure of current drug regimens to effectively treat many diseases. This is especially true for chronic disorders such as the autoimmune inflammatory diseases. The current treatments utilising disease modifying anti-rheumatic drugs (DMARDs) to alleviate

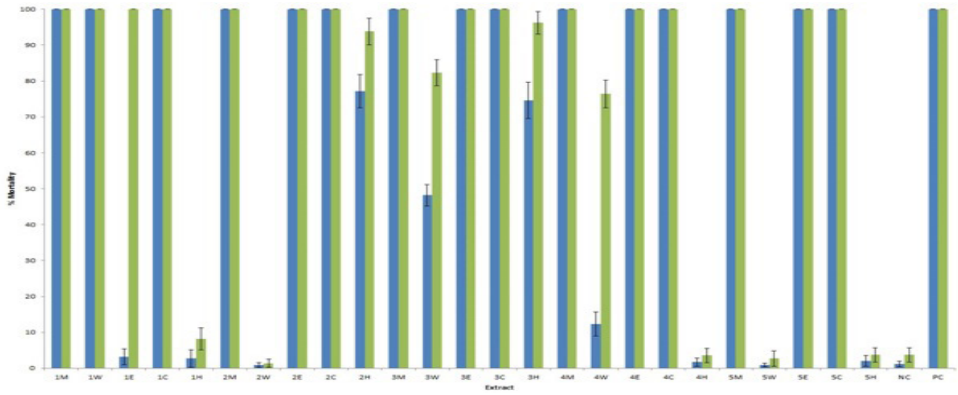

Figure 4: The lethality of the undiluted fruit extracts and the potassium dichromate control (1000 µg/mL) towards Artemia nauplii. The blue bars represent the mortality after 24h exposure and the green bars represent the mortality after 48h exposure. 1=wattle seed; 2=native thyme; 3=native sage; 4=native basil; 5=river mint; M=methanolic extract; M=methanolic extract; W=water extract; E=ethyl acetate extract; C=chloroform extract; H=hexane extract; NC=negative (seawater) control; PC=positive control (1000 µg/ml potassium dichromate). All tests were performed in at least triplicate and the results are expressed as mean ± SEM.

Table 4: The concentrations of the culinary herb extracts required to achieve 50% mortality (LC_{50}) in the *A. franciscana* nauplii assay.

Plant Species	Extract	24 h LC50
Wattle seed	M	5763
	W	6254
	E	-
	C	3230
	H	-
Native Thyme	M	3358
	W	-
	E	1353
	C	3393
	H	1347
Native Sage	M	4015
	W	1831
	E	1550
	C	5477
	H	2480
Native Basil	M	7185
	W	-
	E	1433
	C	8769
	H	-
Rivermint	M	2658
	W	-
	E	186
	C	8254
	H	-
NC		-
PC		186

Values indicate the mean LC50 for triplicate determinations. - indicates that IC50values were not achieved as inhibition did not exceed 50 % at any concentration tested.

the symptoms of these diseases and/or alter the disease progression are not entirely effective and have been associated with numerous adverse effects.[8] Furthermore, many of the current treatments are aimed at treating the symptoms without addressing the underlying causes and pathogenic mechanisms. A better understanding of the mechanisms for initiation and progression of the autoimmune inflammatory diseases is important for developing new drugs to target specific processes and thus more effectively treat autoimmune inflammatory diseases. The studies reported here examined the ability of Australian culinary herb extracts to block microbial triggers of 2 autoimmune inflammatory disorders (Proteus spp.: rheumatoid arthritis; *K. pneumonia*: ankylosing spondylitis). Several species were identified displaying potent inhibition against these bacteria. Particularly noteworthy, the river mint ethyl acetate extract was identified as a potent inhibitor of *K. pneumonia* growth.

A high antioxidant capacity has been postulated as being responsible for the medicinal properties of many plants.[4,30] In particular, antioxidants have been linked to antibacterial, antifungal and antiviral activities, as well as anticancer properties.[4,30] However, other studies have indicated that antioxidants may protect cells from oxidative stress and thus protect against cell death.[31,32] Whilst those studies examined the effects of antioxidants on eukaryotic cells, it is possible that antioxidants may have a similar protective effect against bacterial cell death. Notably, many of the extracts which displayed potent inhibitory activity against the bacterial triggers of rheumatoid arthritis and ankylosing spondylitis had low antioxidant capacities, whilst there was a trend for lower efficacy for the high antioxidant extracts. River mint ethyl acetate extract (0.7 µg ascorbic acid equivalence) was a potent inhibitor of

P. vulgaris and *K. pneumoniae*. In comparison, the methanol extract (10.6 μg ascorbic acid equivalence) did not inhibit *K. pneumoniae* at all and only inhibited *P. vulgaris* at high concentrations. Likewise, the native thyme hexane extract (antioxidant capacity below the threshold of detection) inhibited of the growth of all Proteus strains at substantially lower concentrations than the high antioxidant methanolic extract (11.1 μg ascorbic acid equivalence). Our findings also indicate that only the river mint ethyl acetate extract displayed toxicity towards *Artemia franciscana* (LC50 186 μg/mL). All other extracts were nontoxic. Indeed, all other extracts had LC_{50} values well in excess of 1000 μg/ml.

The phytochemicals responsible for the inhibitory activity of these extracts were not determined in this study. However, alkaloids, anthraquinones, flavonoids, polyphenolics, phytosterols, saponins, stilbenes and terpenes have been linked with anti-bacterial activity in different plant species and thus may be responsible (at least in part) for the bacterial growth inhibitory activities reported here. Several terpenoids have also been reported to suppress NF-κB signaling (the major regulator of inflammatory diseases).[33] The monoterpenes limonene[34,35] and α-pinene[36] have been reported to inhibit NF-κB signaling pathways. α-Pinene affects inflammation by inhibiting p65 translocation into the nucleus in LPS-induced NF-κB signalling.[36] Furthermore, many other sesquiterpenes and sesquiterpene lactones also have well established anti-inflammatory activities.[33] Whilst much work is still needed to characterize the mechanisms of action of these compounds, it appears that NF-κB inhibitory activities may be responsible.

Whilst these studies have demonstrated the potential of several herbs to treat autoimmune disease, much more work is required. This study has only tested these extracts against some microbial triggers of 2 autoimmune diseases (rheumatoid arthritis and ankylosing spondylitis). The microbial triggers for several other autoimmune inflammatory disorders are also known. *Acinetobacter baylyi* and *Pseudomonas aeruginosa* have been linked with the onset of multiple sclerosis[11] and *Borrelia burgdorferi* is linked with Lyme disease.[12] Whilst microbial triggers have also been postulated for lupus, the specific causative agents are yet to be identified. Similarly, members of the Enterobacteriaceae family are associated with Graves' disease and Kawasaki syndrome. *Mycoplasma pneumoniae* is associated with several demyelinating diseases.[13] It would be interesting to extend our studies to also screen for the ability of the extracts to block these microbial triggers of autoimmune diseases.

CONCLUSION

The results of this study demonstrate the potential Australian culinary herb extracts to block the growth of bacterial species associated with the onset of rheumatoid arthritis and ankylosing spondylitis. Further studies aimed at the purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

ACKNOWLEDGEMENTS

Financial support for this work was provided by the Environmental Futures Research Institute and the School of Natural Sciences, Griffith University.

REFERENCES

1. Tapsell LC, Hemphill I, Cobiac L, *et al*. Health benefits of herbs and spices: the past, the present, the future. Med J Aust. 2006; 185(4): S1-24.
2. Anand P, Sundaram C, Jhurani S, *et al*. Curcumin and cancer: an "old-age" disease with an "age-old" solution. Cancer Lett. 2008; 267(1): 133-64.
3. Karalliedde L, Gawarammana I. Traditional herbal medicines. Hammersmith Press London UK; 2008.
4. Cock IE. The phytochemistry and chemotherapeutic potential of *Tasmannia lanceolata* (Tasmanian pepper): A review. Pharmacog Commn. 2013; 3(4): 13-25.
5. Cock IE. Medicinal and aromatic plants – Australia, in Ethnopharmacology section, Biological, Physiological and Health Sciences, Encyclopedia of Life Support Systems (EOLSS), 2011; Developed under the Auspices of the UNESCO, EOLSS Publishers, Oxford, UK, (http://www.eolss.net).
6. LeStrange R. A history of herbal plants. Angus and Robertson; 1977.
7. Netzel M, Netzel G, Tian Q, *et al*. Native Australian fruits–a novel source of antioxidants for food. Innov Food Sci Emerg Technol. 2007; 8(3): 339–46.
8. Alataha D, Kapral T, Smolen JS. Toxicity profiles of traditional disease modifying antirheumatic drugs for rheumatoid arthritis. Ann Rheum Dis. 2003; 62(5): 482-6.
9. Ebringer A, Rashid T. Rheumatoid arthritis is an autoimmune disease triggered by Proteus urinary tract infection. Clin Develop Immunol. 2006; 13(1): 41-8.
10. Ebringer A, Cunningham P, Ahmadi K, *et al*. Sequence similarity between HLA-DR1 and DR4 subtypes associated with rheumatoid arthritis and Proteus/Serratia membrane haemolysins. Ann Rheum Dis. 1992; 51(11): 1245–6.
11. Hughes LE, Smith PA, Natt RS, *et al*. Cross-reactivity between related sequences found in Acinetobacter sp., *Pseudomonas aeruginosa*, myelin basic protein and myelin oligodendrocyte glycoprotein in multiple scherosis. J Neuroimmunol. 2003; 144(1): 105-15.
12. Beermann C, Wunderli-Allenspach H, Groscurth P, *et al*. Lipoproteins from *Borrelia burgdorferi* applied in liposomes and presented to dendritic cells induce CD8+ T-lymphocytes *in vitro*. Cell Immunol. 2000; 201(2): 124-31.
13. Kollef MH, West S, Davis DR, *et al*. Central and peripheral nervous system demyelination after infection with *Mycoplasma pneumoniae*. Evidence of an autoimmune process. Southern Med J. 1991; 84(10): 1255-8.
14. Cock I, van Vuuren SF. Anti-Proteus activity of some South African medicinal plants: their potential for the prevention of rheumatoid arthritis.

Inflammopharmacology 2014; 22(1): 23-36. doi:10.1007/ s10787-013-0179-3
15. Nepom GT, Byers P, Seyfried C, *et al*. HLA genes associated with rheumatoid arthritis. Arth Rheum. 1989; 32(1): 15-21.
16. Wilson C, Ebringer A, Ahmadi K, *et al*. Shared amino acid sequences between major histocompatibility complex class II glycoproteins, type XI collagen and *Proteus mirabilis* in rheumatoid arthritis. Ann Rheum Dis. 1995; 54(3): 216–20.
17. Boyer H, Cock IE. Evaluation of the potential of *Macademia integriflora* extracts as antibacterial food agents. Pharmacog Commn. 2013; 3(3): 53-62.
18. Winnett V, Boyer H, Sirdaarta J, *et al*. The potential of *Tasmannia lanceolata* as a natural preservative and medicinal agent: Antimicrobial activity and toxicity. Pharmacog Commn. 2014; 4(1): 42-52.
19. Jamieson N, Sirdaarta J, Cock IE. The anti-proliferative properties of Australian plants with high antioxidant capacities against cancer cell lines. Pharmacog Commn. 2014; 4(4): 71-82.
20. Mohanty S, Cock IE. Bioactivity of *Syzygium jambos* methanolic extracts: Antibacterial activity and toxicity. Pharmacog Res. 2010; 2(1): 4-9.
21. Kalt FR, Cock IE. GC-MS analysis of bioactive Petalostigma extracts: Toxicity, antibacterial and antiviral activities. Pharmacog Mag. 2014; 10(37 Suppl): S37-48.
22. Vesoul J, Cock IE. The potential of Bunya nut extracts as antibacterial functional food agents. Pharmacog Commn. 2012; 2 (1): 72-9.
23. Cock I, Mohanty S, White A, Whitehouse M. Colloidal silver (CS) as an antiseptic: Two opposing viewpoints. Pharmacog Commn. 2012; 2(1): 49-58.
24. Arkhipov A, Sirdaarta J, Rayan P, *et al.* An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacog Commn. 2014; 4(3): 62-76.
25. Vesoul J, Cock IE. An examination of the medicinal potential of *Pittosporum phylloraeoides*: Toxicity, antibacterial and antifungal activities. Pharmacog Commn. 2011; 1(2): 8-17.
26. Cock IE, van Vuuren SF. The potential of selected South African plants with anti-Klebsiella activity for the treatment and prevention of ankylosing spondylitis. Inflammopharmacology 2014; DOI 10.1007/s10787-014-0222-z.
27. Mpala L, Chikowe G, Cock IE. No evidence of antiseptic properties and low toxicity of selected Aloe species. J Pharm Neg Res. 2010; 1(1): 10-6.
28. Cock IE, Ruebhart DR. Comparison of the brine shrimp nauplii bioassay and the ToxScreen-II test for the detection of toxicity associated with Aloe vera (*Aloe barbadensis* Miller) leaf extract. Pharmacog Res. 2009; 1(2): 102-8.
29. Sautron C, Cock IE. Antimicrobial activity and toxicity of *Syzygium australe* and *Syzygium leuhmanii* fruit extracts. Pharmacog Commn. 2014; 4(1): 53-60.
30. Mohanty S, Cock IE. The chemotherapeutic potential of *Terminalia ferdinandiana*: Phytochemistry and Bioactivity. Pharmacog Rev. 2012; 6(11): 29-36.
31. Montecinos V, Guzmán P, Barra V, Villagrán M, *et al*. Vitamin C is an essential antioxidant that enhances survival of oxidatively stressed human vascular endothelial cells in the presence of a vast molar excess of glutathione. J Biol Chem. 2007; 282(21): 15506-15.
32. Guaiquil VH, Vera JC, Golde DW. Mechanism of vitamin C inhibition of cell death induced by oxidative stress in glutathione-depleted HL-60 cells. J Biol Chem. 2001; 76(44): 40955-61.
33. Salminen A, Lehtonen M, Suuronen T, *et al*. Terpenoids: Natural inhibitors of NF-κB signalling with anti-inflammatory and anticancer potential. Cell Molec Life Sci. 2008; 65(19): 2979-99.
34. Lu XG, Zhan LB, Feng BA, *et al*. Inhibition of growth and metastasis of human gastric cancer implanted in nude mice by d-limonene. World J Gastroenterol. 2004; 10(14): 2140–4.
35. Crowell PL. Prevention and therapy of cancer by dietary monoterpenes. J Nutr. 1999; 129(3): 775S-8S.
36. Zhou JY, Tang FD, Mao GG, *et al*. Effect of a-pinene on nuclear translocation of NF-kB in THP-1 cells. Acta Pharmacol Sin. 2004; 25(4): 480–4.