Pharmacognosy Communications, 2023; 13(3):104-115.
https://www.phcogcommn.org

Original Article

Antimicrobial Activity of *Acacia disparrima* Benth. and *Acacia leiocalyx* Pedley Leaf Extracts in Combination with Antibiotics against Bacterial Triggers of Selected Autoimmune Diseases

Ruxin Guo[1], Xin Yang[1], Ian Edwin Cock[1,2,*]

[1]School of Environment and Science, Griffith University, 170 Kessels Rd, Nathan, Queensland, AUSTRALIA.
[2]Centre for Planetary Health and Food Security, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland, AUSTRALIA.

ABSTRACT

Background: Plants of the genus *Acacia* have been used by Australian Aborigines to treat a variety of conditions including bacterial pathogens and inflammation. Despite this, many *Acacia* spp. have not been evaluated for the ability to inhibit the growth of bacterial triggers of autoimmune inflammatory diseases. This study evaluated the effects of *Acacia disparrima* and *Acacia leiocalyx* leaf extracts alone and in combination against some bacterial triggers of rheumatoid arthritis, ankylosing spondylitis and multiple sclerosis. **Results:** *Acacia disparrima* and *Acacia leiocalyx* leaf extracts displayed noteworthy antibacterial activity against several bacterial triggers of autoimmune diseases. The methanolic extracts were particularly good inhibitors of *P. mirabilis, K. pneumoniae*, and *A. baylyi* with MIC values <1000µg/mL, but were ineffective against *P. aeruginosa*. Furthermore, combining the extracts with conventional antibiotics resulted in significant potentiation of the inhibitory activity for some combinations. Interestingly, all of the synergistic interactions contained tetracycline as the antibiotic component, whilst all of the antagonistic combinations contained either gentamicin or ciprofloxacin as the antibiotic component. None of the individual components (nor the combinations) were toxic in the ALA assay. **Conclusion:** The majority of combinational effects were either additive or indifferent, thereby alleviating some concern related to the concurrent use of *A. disparrima* and *A. leiocalyx* whilst also taking conventional antibiotics. A few notable combinations were identified, indicating the need for further *in vivo* testing.

Keywords: Australian plants, Medicinal plants, Synergy, Rheumatoid arthritis, Ankylosing spondylitis, Multiple sclerosis, Drug combinations, Efflux pump inhibitor.

Correspondence:
Dr. Ian Edwin Cock[1,2]
[1]School of Environment and Science, Griffith University, 170 Kessels Rd, Nathan, Queensland, 4111, AUSTRALIA.
[2]Centre for Planetary Health and Food Security, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland, 4111, AUSTRALIA.
Email: i.cock@griffith.edu.au

Received: 04-04-2023;
Revised: 26-04-2023;
Accepted: 20-05-2023.

INTRODUCTION

Specific exogenous antigens may trigger autoimmune inflammatory disorders such as ankylosing spondylitis, multiple sclerosis, rheumatic fever and rheumatoid arthritis in genetically susceptible people. In total, approximately 80 autoimmune diseases with varying susceptibility profiles have been reported.[1,2] For many of these diseases, the triggers are established, the etiological events of other autoimmune diseases remain unknown. Whilst some of these diseases are triggered by environmental and dietary stimuli, the majority are triggered by bacterial and viral pathogens. There are currently no cures available for any of these diseases. Instead, the therapeutic approach is administer anti-inflammatory drugs and analgesics to treat the symptoms. Whilst this approach decreases the patient's discomfort, tissue damage can still occur. Additionally, the prolonged used of anti-inflammatory drugs causes toxicity and numerous deleterious side effects.[3] Instead, targeting the trigger antigens of autoimmune diseases may block the disease etiology, thereby preventing the diseases and its downstream effects. Many of the pathogenic triggers of these diseases have already been identified by serotyping studies, allowing the use chemotherapies targeting the diseases prevention.[2] *Proteus mirabilis* can trigger rheumatoid arthritis in genetically susceptible people,[4] whilst *Klebsiella pneumoniae* may trigger ankylosing spondylitis.[5] Multiple sclerosis can be initiated in genetically susceptible people by *Acinetobacter baylyi* and *Pseudomonas aeruginosa*

DOI: 10.5530/pc.2023.3.18

infections,[6] and *Streptococcus pyogenes* may induce the onset of rheumatic fever.[7]

Prophylactic antibiotic therapies targeting these pathogens is an attractive option as it would prevent the disease onset, and thus the downstream symptoms. However, prolonged prophylactic antibiotic monotherapy use would result in the development bacterial resistance towards the therapy, decreasing the efficacy of the antibiotic for future use.[8] Indeed, the misuse and overuse of antibiotics has already resulted in the development of bacterial pathogens with resistance to multiple antibiotic therapies.[8] These multi-resistant pathogens are becoming increasingly difficult to manage using the current range antibiotic chemotherapies and previously effective therapies are failing to address some infections.[8,9] This has resulted in dramatic increases in the mortality associated with some bacterial pathogens.[10] Furthermore, the discovery of new antimicrobial agents has rapidly declined in recent years, and few antibiotics synthesised or discovered in the last decade were introduced clinically during that period.[8] The development of safe and effective new antibiotic therapies is crucial and is considered by the World Health Organisation (WHO) to be perhaps the most urgent challenge facing medical science.[11] For a number of reasons reviewed elsewhere,[8] the current methods of antibiotic discovery/development are unlikely to provide an adequate pipeline of new antibiotics in the future and alternative drug discovery and treatment modalities may be required.

A re-examination of ethnobotany and traditional plant-based medicines is a promising approach for antimicrobial drug development. Plant-derived medicines were commonly used to treat pathogenic disease prior to the modern era of antibiotic discovery, which began with the discovery of penicillin.[8] Ancient cultures had a good understanding of plant species with medicinal properties and much of that traditional knowledge was associated with the treatment and eradication of bacterial pathogens.[8] Indeed, several traditional healing systems (e.g. Ayuveda and Traditional Chinese Medicine (TCM)) are still in common use and are often well documented, making species selection for screening relatively simple. Furthermore, some plant medicines contain multiple well characterised antibacterial compounds. The presence of multiple antibacterial compounds (which often function via different mechanisms) not only increases the efficacy of the therapy, but also decreases the possibility of the therapy inducing further bacterial resistance.[8]

Despite the widespread use of traditional medicines, surprisingly few plant preparations or isolated compounds are commonly used as antimicrobial therapies in allopathic medicine. This may be due to the preference for monotherapies by allopathic medicine. Traditional medicines often require synergistic interactions between components in the formulation to potentiate the antibacterial activity of the medicine. Purified compounds may be substantially less potent than the crude extract they are derived from.[12] A combinational therapy model that utilises synergistic interactions between conventional antibiotics and plant extracts (or pure plant compounds) may therefore be more effective for the treatment of bacterial diseases, especially against antibiotic resistant bacterial strains.[13-15] Indeed, combinational therapies are already preferred over mono-therapies for the treatment of several life-threatening infectious diseases including malaria, tuberculosis and HIV/AIDS as different components in the combinational therapy target multiple facets of a disease, thereby increasing the efficacy of the therapy and reducing the development of further resistance.[8,12] As the development of new drugs may require years of costly clinical trials, therapies containing combinations of conventional antibiotics and plant extracts/isolated compounds may also have economic advantages.[12] Combinational therapies may potentially block bacterial resistance mechanisms, thereby restoring the efficacy of an antibiotic therapy.

The genus *Acacia* (family *Fabaceae*, subfamily *Mimosaceae*) consists of over 1200 species of which more than 700 are indigenous to Australia.[16] Other species are spread throughout tropical to warm temperate regions of Africa, India and the Americas. *Acacias* have also been introduced globally for ornamental and economic purposes. Most *Acacia* species produce quality wood and some are also valuable sources of proteins, tannins, gum, perfumes, paint, ink and flavouring agents.[17,18] Furthermore, *Acacia* seed formed an important part of the diet for Australian Aborigines as an easily obtainable, high-energy food.[19,20] *Acacia* seed can easily be ground to a flour which is then mixed with water and eaten either raw or cooked to produce an unleavened bread. Powdered *Acacia* seed flour inhibits the growth of several species of food spoilage bacteria and thus has potential as natural food preservatives.[21] Other parts of some *Acacia* species are also eaten. Several species exude a sugary gum from wounds to the stem and branches[17,20] whilst others are hosts for edible grubs often referred to as witchetty grubs by non-Aboriginal Australians.[22]

Australian *Acacia* species were also used as traditional bush medicines by the first Australians. Several species were used to prepare antimicrobial washes and lotions.[23] Unfortunately most of our understanding of the antimicrobial potential of Australian *Acacia* species is anecdotal with few species being rigorously studied. These anecdotal accounts demonstrate that the first Australians knew of the antibacterial properties of the Australian *Acacia* spp. and used them for an array of therapeutic purposes to treat many diseases. Recent studies[23,24] have demonstrated the antibacterial activity of methanolic extracts of several species of Australian *Acacia* against a limited panel of bacteria. However, the therapeutic properties of many other Australian *Acacia* spp. are yet to be investigated. The current study was undertaken to assess the growth inhibitory properties of two species of Australian *Acacia* spp. (*Acacia disparrima* Benth. and *Acacia leiocalyx* Pedley) against of a panel of bacterial pathogens that

trigger some autoimmune inflammatory diseases.[1,2] Furthermore, the interactive antimicrobial and toxicity profiles of combinations of the *A. disparrima* and *A. leiocalyx* leaf extracts and six conventional antibiotic drugs was examined.

MATERIALS AND METHODS

Sourcing and preparation of plant samples

Acacia disparrima Benth. and *Acacia leiocalyx* Pedley material was provided and identified by Professor Xu Zihong of Griffith University. The leaves were sourced from Toohey Forest, Brisbane, Australia and voucher specimens are deposited in the School of Environment and Science, Griffith University, Australia (voucher numbers ADL1A-2016-A and ALL1A-2016A respectively). Individual 1g masses of the ground plant material were weighed into separate tubes and 50 mL of methanol, deionised water, ethyl acetate chloroform or hexane were added. All solvents were obtained from Ajax Fine Chemicals, Australia and were AR grade. The ground plant materials were extracted in each solvent for 24 hr at 4°C by gentle shaking. The extracts were subsequently filtered through filter paper (Whatman No. 54) under vacuum. The solvent extracts were air dried at room temperature in the shade. The aqueous extracts were lyophilised by freeze drying at -50°C. The resultant dried extracts were weighed and dissolved in 10 mL deionised water (containing 1% DMSO).

Qualitative phytochemical analysis

Phytochemical analysis of the *A. disparrima* and *A. leiocalyx* leaf extracts for the presence of cardiac glycosides, alkaloids, saponins, tannins, flavonoids, phenolic compounds, phytosterols, flavonoids and triterpenoids were achieved as previously described.[25,26]

Antibacterial analysis

Conventional antibiotics

Penicillin-G (potency of 1440-1680 µg/mg), chloramphenicol (≥98% purity by HPLC, erythromycin (potency ≥850µg/mg), gentamicin (potency of 600µg/mg), and tetracycline (≥95% purity by HPLC) were purchased from Sigma-Aldrich, Australia and used for the microplate liquid dilution assay. All antibiotics were prepared in sterile deionised water at stock concentrations of 0.01 mg/mL and stored at 4°C until use. For the disc diffusion studies, ampicillin (2µg) and chloramphenicol discs (10µg) standard discs were obtained from Oxoid Ltd., Australia and used as positive controls.

Bacterial cultures

All bacterial strains were selected based on their ability to trigger autoimmune inflammatory diseases in genetically susceptible individuals.[27] Reference strains of *Proteus mirabilis* (ATCC21721), *Klebsiella pneumoniae* (ATCC31488), *Acinetobacter baylyi* (ATCC33304) and *Pseudomonas aeruginosa* (ATCC39324) were purchased from American Type Culture Collection, USA. A clinical isolate strain of *Streptococcus pyogenes* was obtained from the School of Natural Sciences teaching laboratory, Griffith University, Australia. All bacteria were cultured in nutrient broth (Oxoid Ltd., Australia). Streak nutrient agar (Oxoid Ltd., Australia) plates were tested in parallel to ensure the purity of all bacterial cultures and for sub-culturing. All bacterial cultures were incubated at 37°C for 24 hr and were subcultured and maintained in nutrient broth at 4°C until use.

Evaluation of bacterial susceptibility to growth inhibition

The susceptibility of the bacteria to the *A. disparrima* and *A. leiocalyx* leaf extracts and the conventional antibiotics was initially assessed using a modified disc diffusion assay.[28,29] Ampicillin (2µg) and chloramphenicol discs (10µg) were obtained from Oxoid Ltd., Australia and used as positive controls to compare antibacterial activity. Filter discs infused with 10 µL of distilled water were used as a negative control.

Minimum Inhibitory Concentration (MIC) determination

The minimum inhibitory concentration for each extract was determined using two methods. A liquid dilution MIC assay was employed as it is generally considered the most sensitive bacterial growth inhibitory assay.[30,31] Furthermore, as microplate liquid dilution MIC assays are perhaps the most commonly used method of quantifying bacterial growth inhibition efficacy, use of this method allows for comparisons with other studies. A solid phase agar disc diffusion assay was also used in this study for comparison.

Microplate Liquid Dilution MIC assay

A standard liquid dilution MIC 30 was used to evaluate the antimicrobial activity of the plant samples and the conventional antimicrobials independently and in combinations. Briefly, 100µL of sterilized distilled water was dispensed into each well of 96 well micro-titre plate. The plant samples and conventional antibiotics (100µL) were then added into separate wells of the first row of the plate. Plant samples were introduced at a starting concentration of 32mg/mL whilst the conventional antibiotics were introduced at a starting concentration of 0.01mg/mL. A negative control (nutrient broth), a sterile control (without bacteria) and a sample-free culture control (to ensure the media was capable of supporting microbial growth) were included on all plates. After addition of the test samples to the plate, each was serially diluted by doubling dilution. The relevant bacterial culture inoculum (100µL) was then added to all wells of the plate except the sterile control wells. Each inoculum contained approximately 1×10^6

Colony Forming Units (CFU)/mL. All plates were subsequently incubated at 37°C. p-Iodonitrotetrazolium violet (INT) was obtained from Sigma-Aldrich, Australia and dissolved in sterile deionised water to prepare a 0.2mg/mL INT solution. A 40µL volume of this solution was added into all wells and the plates were incubated for a further 6 hr at 30°C. Following incubation, the MIC was visually determined as the lowest dose at which colour development was inhibited.

Disc diffusion MIC assay

The Minimum Inhibitory Concentrations (MIC) of the extracts was also evaluated by disc diffusion assay as previously described.[32,33] Graphs of the Zone of Inhibition (ZOI) versus Ln of the concentration were plotted and MIC values were achieved using linear regression.

Extract-conventional antibiotic interaction studies

Fractional Inhibitory Concentration (FIC) assessment

Interactions between the combinations of plant samples and conventional antimicrobials were further classified using the sum of the Fractional Inhibitory Concentration (ΣFIC). The FIC was calculated using the following equation, where (a) represents the plant sample and (b) the conventional antimicrobial sample 12:

$$FIC^{(i)} = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}}$$

$$FIC^{(ii)} = \frac{\text{MIC (b) in combination with (a)}}{\text{MIC (b) independently}}$$

The ΣFIC was then calculated using the equation: $\Sigma FIC = FIC^{(i)} + FIC^{(ii)}$. The interactions were classified as being synergistic for ΣFIC values of ≤0.5, additive (>0.5–1.0), indifferent (>1.0–≤ 4.0) or antagonistic (>4.0).[12] Tentative interpretations were included where the MIC value was greater than the highest concentration tested to provide an estimation of what the possible interactive profile for the combination could have been. These interpretations were not given a ΣFIC value, as only absolute values could be used in ΣFIC calculations and not 'greater than' values.

Varied ratio combination studies (isobolograms)

Where a synergistic interaction was identified, it was further evaluated at different ratios to determine the best ratios for therapeutic use. Nine combinations containing 10 to 90% (extract) were tested. All ratios were tested in duplicate in two independent experiments (n=4). And the data is presented as the mean of four replicates. Mean data points for each ratio were plotted on an isobologram and analysed to determine the optimal combination ratios to obtain synergy. Data points on or below the 0.5:0.5 line on the isobologram indicated synergy; data points above the 0.5:0.5 line, up to and including the 1.0:1.0 line indicated an additive interaction; data points above the 1.0:1.0 line indicated non-interactive combination ratios.

Brine-shrimp lethality assay

The toxicity of the *A. disparrima* and *A. leiocalyx* leaf extracts, conventional antibiotics and the reference toxin were assessed using a modified *Artemia franciscana* nauplii Lethality Assay (ALA).[34,35] Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared in deionised water (4 mg/mL) and serially diluted in artificial seawater as a reference toxin. The mortality induction of all tests and controls was assessed at 24 and 48 hr and expressed as a % of the untreated control. The LC_{50} for each treatment was calculated using Probit analysis.

Statistical analysis

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

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extractions of the *A. disparrima* and *A. leiocalyx* leaf extracts (1 g) with solvents of varying polarity yielded dried plant extracts ranging from 65mg (*A. leiocalyx* leaf hexane extract) to 301mg (methanolic *A. disparrima* leaf extract) (Table 1). Qualitative phytochemical screening (Table 1) showed that the higher polarity solvents (methanol and water) extracted the greatest amount and widest diversity of phytochemical classes.

Bacterial growth inhibition screening

Proteus mirabilis growth was particularly susceptible to the higher polarity aqueous and methanolic *A. disparrima* and *A. leiocalyx* leaf extracts and were unaffected by the lower polarity extracts (Figure 1a). Indeed, Zones of Inhibition (ZOIs) of approximately 9.8 and 7.8 mm were recorded for the methanolic and aqueous extracts *A. disparrima* respectively against *P. mirabilis,* compared with ZOIs of 8.4 and 6.6 mm for the *A. leiocalyx* methanolic and aqueous extracts. Notably, these methanolic extracts produced ZOIs comparable with the reference antibiotics ampicillin and tetracycline (9.3 and 8.4mm respectively). In contrast, the chloramphenicol control was a strong inhibitor of *P. mirabilis* growth, with a ZOI of 13.8mm. The lower polarity chloroform and hexane extracts were completely devoid of inhibitory activity, indicating that the major antibacterial components in *A. disparrima* and *A. leiocalyx* leaves may be polar. Similarly, the methanolic and aqueous *A. disparrima* and *A. leiocalyx* leaf

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the *Acacia* spp. leaf extracts.

Plant species	Extract	Mass of Dried Extracted Material (mg)	Concentration of extract (mg/mL)	Phenols			Cardiac Glycosides	Saponins	Triterpenes	Phytosterols	Alkaloids		Flavonoids		Tannins	Anthraquinones	
				Total Phenolics	Water Soluble	Water Insoluble	Keller-Kiliani Test	Froth Persistence	Salkowski Test	Acetic Anhydride Test	Meyers Test	Wagners Test	Shinoda Test	Kumar test	Ferric Chloride Test	Free	Combined
Acacia diaparrima	Methanol	301	30.1	+++	+++	++	-	++	-	+	-	-	+++	++	+++	-	-
	Water	142	14.2	+++	+++	+	-	++	-	-	-	-	+++	++	+++	-	-
	Ethyl Acetate	61	6.1	++	++	-	-	-	-	-	-	-	+	+	+	-	-
	Chloroform	138	13.8	+	+	-	-	-	+	-	-	-	-	-	-	-	-
	Hexane	78	7.8	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Acacia leiocalyx	Methanol	251	25.1	+++	+++	++	-	++	-	-	-	-	+++	++	+++	-	-
	Water	126	12.6	+++	+++	+	-	++	-	-	-	-	+++	++	+++	-	-
	Ethyl Acetate	79	76.9	++	++	-	-	+	-	-	-	-	++	+	++	-	-
	Chloroform	122	12.2	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	Hexane	65	6.5	-	-	-	-	-	+	-	-	-	-	-	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay.

extracts also inhibited the growth of *K. pneumoniae* (Figure 1b) albeit, generally with substantially lower efficacy than for *P. mirabilis* growth inhibition. The *A. leiocalyx* leaf extracts were better growth inhibitors of *K. pneumoniae* (ZOIs of 7.9 and 8.3mm for the methanolic and aqueous extracts respectively), than the *A. disparrima* extracts (ZOIs = 7.3 and 7.6mm respectively). The noteworthy growth inhibitory activity of the methanolic and aqueous *Acacia* spp. leaf extracts against both *P. mirabilis* (a bacterial trigger of rheumatoid arthritis) and *K. pneumoniae* (a trigger of ankylosing spondylitis) indicate that they may be useful for the prevention and treatment of these diseases, as well as other diseases that these bacteria cause.

The methanolic *A. disparrima* and *A. leiocalyx* leaf extracts were also better inhibitors of the growth of *A. baylyi* (a bacterial trigger of multiple sclerosis in genetically susceptible people) than the corresponding aqueous extracts, with a ZOIs of 8 and 7.3mm respectively (compared to 7.2 and 6.8 mm for the corresponding aqueous extracts) (Figure 1c). In contrast, the chloramphenicol and tetracycline controls produced relatively large ZOIs (11.4 and 9.5mm respectively). The ampicillin control was a less potent *A. baylyi* inhibitor (on the basis of ZOI), with a 7.2mm ZOI. In contrast, all *A. disparrima* and *A. leiocalyx* leaf were completely ineffective against *P. aeruginosa* (another bacterial trigger of multiple sclerosis) (Figure 1d). It is noteworthy that the *P. aeruginosa* strain tested in this study was a highly resistant strain and was also completely resistant to both the ampicillin

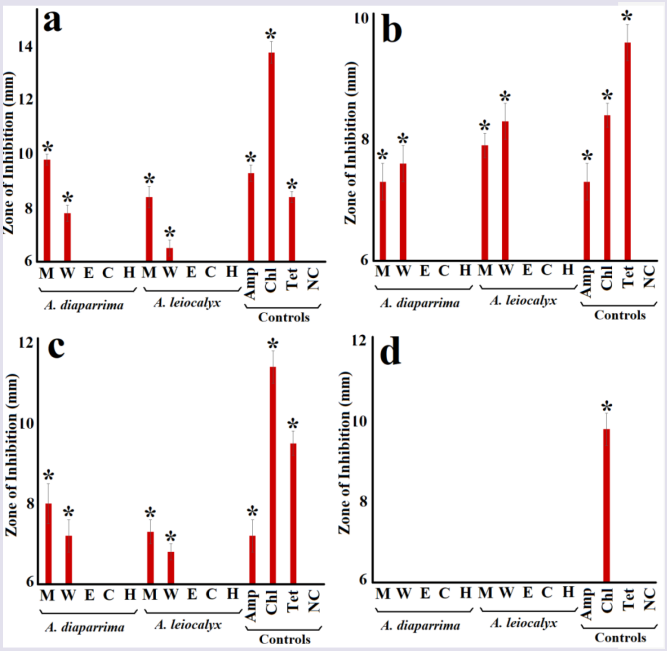

Figure 1: Antibacterial activity of *A. disparrima* and *A. leiocalyx* leaf extracts against (a) *P. mirabilis* (ATCC21721); (b) *K. pneumoniae* (ATCC31488); (c) *A. baylyi* (ATCC33304); and (d) *P. aeruginosa* (ATCC: 39324), measured as zones of inhibition (mm). M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (2µg); Chl = chloramphenicol (10µg); Tet = tetracycline (10µg); NC = negative control (nutrient broth). Results are expressed as mean zones of inhibition of at least six replicates (two repeats) ± SEM * indicates results that are significantly different to the negative control (p<0.01).

and tetracycline controls. Our studies therefore indicate that the methanolic *A. disparrima* leaf extract (and to a lesser extent, the methanolic *A. leiocalyx* leaf extract) were the most effective inhibitor of the bacterial triggers of multiple sclerosis.

Quantification of Minimum Inhibitory Concentration (MIC)

The relative level of antimicrobial activity was further evaluated by determining the MIC values using two methods: the liquid dilution MIC assay and the disc diffusion MIC assay (Table 2). Consistent with the antibacterial screening assays, of the methanol *A. disparrima*. and *A. leiocalyx* leaf extracts inhibited the bacteria tested (with the exception of *P. aeruginosa*) and they were generally more potent bacterial growth inhibitors than the corresponding aqueous extracts. Also similar to the disc diffusion susceptibility studies, the ethyl acetate, chloroform and hexane were completely devoid of inhibitory activity, indicating that the antibacterial components are polar. The MIC values of the conventional antibiotic controls were only determined for the liquid dilution assay as commercial discs containing a fixed mass of antibiotic were used in the disc diffusion assay. Thus, the zones of only single doses were recorded for that assay and we were unable to determine MIC values. Gentamicin was the most potent antibiotic (as judged by its MIC). Indeed, most of the bacterial strains tested were partially resistant to all of the conventional antibiotics, although only *P. mirabilis* was partially resistant to gentamicin.

The MIC values determined for *A. disparrima*. and *A. leiocalyx* leaf extracts compare relatively well between the disc diffusion and liquid dilution assays. The growth of *P. mirabilis* was strongly inhibited by the methanolic *A. disparrima*. and *A. leiocalyx* leaf extract (LD MIC's of 110 µg/mL and 311 µg/mL respectively). The aqueous *A. disparrima*. and *A. leiocalyx* extracts (LD MIC's of 378µg/mL and 695µg/mL were also noteworthy *P. mirabilis* growth inhibitors. The *disparrima*. and *A. leiocalyx* aqueous leaf extracts also inhibited *P. mirabilis* growth with noteworthy activity (LD MIC's of 378µg/mL and 311µg/mL respectively). Similarly, the methanolic and aqueous *A. disparrima*. and *A. leiocalyx* leaf extracts were noteworthy inibitors of *K. pneumoniae* growth. MIC values of 994 and 932µg/mL were recorded for the methanolic extracts against this bacterium respectively, compared to 746 and 585µg/mL for the corresponding aqueous extracts. additionally, the methanolic and aqueous *A. disparrima* and *A. leiocalyx* leaf extracts were moderate to good inhibitors of *A. baylyi* growth (methanolic extract MIC values + 730 and 328µg/mL respectively; MIC values = 1468 and 725µg/mL for the aqueous extracts). As note for the disc diffusion assay, all extracts wetre completely ineffective against *P. aeruginosa*.

Combinational effects: Fractional Inhibitory Concentration (FIC) assessment

Three combinations of the *A. disparrima* and *A. leiocalyx* leaf extract with the conventional antibiotic combinations produced synergy in the combinational assays. Notably, synergy was only detected when tetracycline was the antibiotic component of the combination, indicating that the extracts may have phytoconstituents that block bacterial tetracycline efflux pump (the major bacterial tetracycline resistance mechanism).[8] Specifically, combinations containing the methanolic extracts of either *Acacia* spp. and tetracycline were synergistic against *P. mirabilis*. Similarly, the *A. leiocalyx* leaf extract-tetracycline was also synergistic. Therefore, these combinations have substantially potentiated antibacterial effects against these bacterial pathogens and may be particularly beneficial for the treatment and prevention of rheumatoid arthritis and multiple sclerosis.

Additionally, five (~21%) of the *Acacia* spp. extract-antibiotic combinations produced additive effects when tested against each of the *P. mirabilis, K. pneumoniae* and *A. baylyi* strains (Table 3). As these combinations also produce effects greater than either the individual extract or conventional antibiotic components, these combinations would be beneficial in the prevention and treatment of rheumatoid arthritis. The majority

Table 2: Disc Diffusion (DD) and Liquid Dilution (LD) MIC values (µg/mL) for *Acacia* spp. leaf extracts against microbial triggers of some autoimmune inflammatory diseases.

Extract		*P. mirabilis*		*K. pneumoniae*		*A. baylyi*		*P. aeruginosa*	
		DD MIC	LD MIC	DD MIC	LD MIC	DD MIC	LD MIC	DD MIC	LD MIC
A. diaparrima	M	256	110	1452	994	1110	730	-	-
	W	827	378	1386	746	2500	1468	-	-
	E	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-
	H	-	-	-	-	-	-	-	-
A. leiocalyx	M	656	311	1260	932	556	328	-	-
	W	1083	695	896	585	1180	725	-	-
	E	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-
	H	-	-	-	-	-	-	-	-
Controls	Pen	-	1.25	-	1.9	-	2.5	-	1.25
	Chl	-	3.3	-	1.9	-	1.9	-	0.3
	Eryth	-	2.5	-	1.9	-	1.25	-	1.25
	Tet	-	0.63	-	0.3	-	1.25	-	1.25
	Gent	-	1.25	-	0.31	-	0.31	-	0.63
	Cip	-	0.63	-	0.63	-	0.63	-	1.25
	NC	-	-	-	-	-	-	-	-

M = methanol extract; W = water extract; E = ethyl acetate extract; C = chloroform extract; H = hexane; DD = disc diffusion; LD = liquid dilution; Pen = penicillin-G; Chlor = chloramphenicol; Eryth = erythromycin; Tet = tetracycline; Gent = gentamycin; Cip = ciprofloxacin. - indicates no inhibition at any dose tested.

(16 of the 24 combinations: ~67%) of the other combinations were non-interactive. Whilst these combinations provide no added benefit over that of the individual components alone, the components do not antagonise each other's effects and are therefore safe to use concurrently without risk of lessening the efficacy of either component. This is a very important finding as many people self-medicate with herbal medicines without their medical practitioner's knowledge. Therefore, it is very important to determine where drug interactions occur, affecting the efficacy of the conventional antibiotic.

However, five combinations of the *Acacia* spp. extracts and conventional antibiotics were antagonistic. This is important information as it indicates that these combinations should be avoided for clinical usage. Interestingly, nearly all of the antagonistic combinations contained gentamicin as the antibiotic component. This is perhaps not surprising as gentamicin is still a very useful antibiotic as relatively few bacteria have yet developed resistance to it.[8] One combination with ciprofloxacin (with *A. leiocalyx* leaf methanolic extract against *K. pneumoniae*) was also antagonistic. Two other trends were also evident: antagonism only occurred when the extract component was the methanolic extract of either *Acacia* species, and that antagonism only occurred when tested against *K. pneumoniae* and *A. baylyi*. Therefore, all extract-antibiotic combinations are generally safe to use to prevent and treat rheumatoid arthritis (which may be triggered by *P. mirabilis*) without negatively impacting the effectiveness of the therapy. In contrast, combinations containing gentamicin and the *Acacia* spp. methanolic extracts should be avoided for the prevention and treatment of ankylosing spondylitis and multiple sclerosis (with may be triggered in genetically susceptible by *K. pneumoniae* and *A. baylyi* respectively).

Varied ratio combination studies (isobolograms)

Three synergistic combinations were detected in the combinational studies. Notably, all of these combinations included tetracycline as the conventional antibiotic component. These combinations were further examined using isobologram analysis across a range of extract:tetracycline ratios to identify the ideal ratios to obtain synergy (Figure 2). In general, >30% extract component was required for synergy (although only >20% extract was required for the tetracycline-*A. diaparrima* methanolic extract against *P. mirabilis*). Additionally, there was no clear correlation of the ratio effects with either axis. Isobologram such as these are indicative of competition between the conventional antibiotic and an extract component for the bacterial resistance mechanism.[8] As the extract component of the combination increases in ratio, it

Table 3: ΣFIC values for the *Acacia* spp. leaf extract and conventional antibiotic combinations against susceptible bacteria.

Bacteria	Extract	Pen	Chl	Eryth	Tet	Gent	Cip
P. mirabilis	AD M	*0.738*	2.86	*0.63*	0.38	2.45	2.86
		(ADD)	(IND)	*(ADD)*	(SYN)	(IND)	(IND)
	AD W	1.14	1.84	0.85	*0.58*	3.35	3.75
		(IND)	(IND)	(ADD)	*(ADD)*	(IND)	(IND)
	AL M	1.17	1.56	*0.98*	0.43	1.86	2.78
		(IND)	(IND)	*(ADD)*	(SYN)	(IND)	(IND)
	AL W	1.46	1.76	1.44	*0.66*	2.73	3.24
		(IND)	(IND)	(IND)	*(ADD)*	(IND)	(IND)
K. pneumoniae	AD M	*0.624*	1.18	*0.68*	*0.94*	4.16	3.64
		(ADD)	(IND)	*(ADD)*	*(ADD)*	(ANT)	(INT)
	AD W	1.25	3.26	2.84	1.86	3.28	2.88
		(IND)	(IND)	(IND)	(IND)	(IND)	(INT)
	AL M	*0.845*	1.03	*0.87*	1.24	4.65	4.13
		(ADD)	(IND)	*(ADD)*	(IND)	(ANT)	(ANT)
	AL W	1.33	2.84	2.25	2.25	3.72	3.16
		(IND)	(IND)	(IND)	(IND)	(IND)	(IND)
A. baylyi	AD M	*0.836*	1.18	1.12	*0.63*	4.92	3.74
		(ADD)	(IND)	(IND)	*(ADD)*	(ANT)	(IND)
	AD W	1.02	2.63	1.68	1.32	2.88	3.45
		(IND)	(IND)	(IND)	(IND)	(IND)	(IND)
	AL M	1.06	*0.88*	*0.68*	0.36	4.22	2.95
		(IND)	*(ADD)*	*(ADD)*	(SYN)	(ANT)	(IND)
	AL W	1.37	1.18	*0.92*	1.28	2.9	2.42
		(IND)	(IND)	*(ADD)*	(IND)	(IND)	(IND)

M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; H = hexane extract; SYN = synergistic interaction; *ADD = additive interaction*; IND = indifferent interaction: ANT = antagonistic interaction.

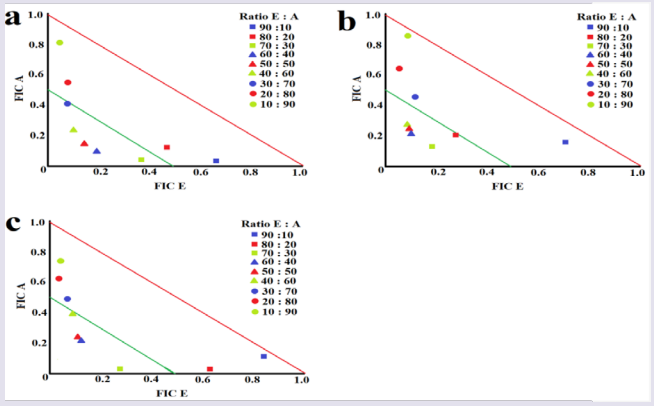

Figure 2: Isobologram for combinations of tetracycline with (a) *A. diaparrima* methanolic extract against *P. mirabilis*, (b) *A. leiocalyx* methanolic extract against *P. mirabilis* and (c) *A. leiocalyx* methanolic extract against *A. baylyi*. All combinations were tested at various ratios and results represent mean MIC values of four replicates. Ratio = % extract: % antibiotic. Ratios lying on or underneath the 0.5:0.5 line are considered to be synergistic (Σ FIC ≤ 0.5). Any points between the 0.5:0.5 and 1.0:1.0 lines are deemed additive (Σ FIC > 0.5-1.0).

outcompetes the antibiotic, thereby blocking its inactivation and allowing it to be functional again, even in otherwise resistant bacteria.

As combinations containing <80% extract was synergistic, with all ratios outside this range being additive. Thus, all ratios of these combinations containing 30-80% extract would be beneficial to enhance *P. mirabilis* and *A. baylyi* growth inhibition. However, bacteria would be less likely to develop resistance when combinations are used in ratios which minimise the amount of conventional antibiotic used. Thus, for long term prophylactic treatment (as would be required to prevent and treat rheumatoid arthritis and multiple sclerosis), the ideal extract:tetracycline ratio may be 80:20. However, when used for the treatment of acute infections (e.g., urinary tract infections), the ratio which maximises the efficacy of the treatment (i.e., the 20:80 ratio) may be the preferred option.

Table 4: Mortality (%) assessment for extracts and conventional antibiotics tested individually and as combinations in the *Artemia* lethality assay.

	Sample	Mortality ± SD (%)	
		After 24 hr	After 48 hr
Antimicrobials	Penicillin G	1.8 ± 1.4	4.3 ± 2.4
	Chloranphenicol	2.7 ± 1.3	5.6 ± 3.3
	Erythromycin	1.2 ± 0.6	5.8 ± 2.3
	Tetracycline	2.4 ± 1.5	5.1 ± 2.8
	Gentamicin	3.1 ± 1.8	6.7 ± 2.6
	Ciproflaxacin	5.5 ± 2.0	8.3 ± 2.1
Extracts	AD M	6.7 ± 3.2	22.4 ± 3.6
	AD W	7.4 ± 2.6	19.8 ± 2.8
	AL M	5.3 ± 2.4	16.5 ± 2.2
	AL W	6.8 ± 2.9	18.5 ± 3.4
Combinations	AD M + Pen	7.4 ± 4.2	31 ± 3.7
	AD W + Pen	6.3 ± 2.4	24.5 ± 4.5
	AL M + Pen	8.4 ± 2.9	26.5 ± 3.7
	AL W + Pen	9.6 ± 3	25.1 ± 4.2
	AD M + Chl	9.7 ± 2.7	33.4 ± 3.6
	AD W + Chl	8.2 ± 3.2	26.8 ± 4.1
	AL M + Chl	10.7 ± 3.6	29.3 ± 4.6
	AL W + Chl	10.2 ± 3.6	30.2 ± 3.6
	AD M + Eryth	7.2 ± 3.8	17.6 ± 3.1
	AD W + Eryth	8.5 ± 3.6	24.4 ± 3.9
	AL M + Eryth	6.4 ± 3.1	14.9 ± 3.5
	AL W + Eryth	9.2 ± 4	21.7 ± 3.8
	AD M + Tet	6.5 ± 2.7	20.1 ± 3.2
	AD W + Tet	8.3 ± 3.2	26.4 ± 3.3
	AL M + Tet	6.6 ± 4.6	17.5 ± 4.1
	AL W + Tet	8.8 ± 3.4	24.8 ± 3.2
	AD M + Gen	9.7 ± 2.8	29.8 ± 4.6
	AD W + Gen	7.3 ± 3.8	29.6 ± 3.7
	AL M + Gen	8.4 ± 3.5	22.7 ± 4
	AL W + Gen	11.8 ±3.4	37.5 ± 4.5
	AD M + Cip	8.5 ± 3.6	26.4 ± 4.7
	AD W + Cip	9.2 ± 3.7	21.1 ± 3.9
	AL M + Cip	11.3 ± 4.2	29.2 ± 3.4
	AL W + Cip	10.4 ± 3.7	27.6 ± 3.8
Controls	Deionised water	2.7 ± 1.7	3.6 ± 2.5
	Potassium dichromate	100.00 ± 0.00	

Potassium dichromate was tested at a concentration of 1000µg/mL; M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; H = hexane extract; Chl = chloramphenicol; Gent = gentamicin; Cip = ciprofloxacin; SD = standard deviation. Results represent means ± SEM of 3 independent experiments, each preformed in triplicate (n = 9).

Toxicity studies

All plant extracts and antibiotics were individually screened at 1mg/mL in the *Artemia* Lethality Assay (ALA). The extracts were only considered toxic if they induced percentage mortalities greater than 50% (LD_{50}) following 24 hr of exposure to the *Artemia* nauplii 35. When tested individually, the antimicrobials demonstrated no toxicity in the ALA (Table 4). Similarly, none of the *L. salicaria* extracts produced mortality or cell viability significantly different to that of the negative control. When tested in together in the ALA, none of the extract-antibiotic combinations produced mortality significantly different to the negative controls, and no single component nor combination induced >50% mortality. Therefore, all combinations and individual components were deemed nontoxic. In contrast, the positive control potassium dichromate induced 100% mortality in the ALA.

DISCUSSION

This study investigated the ability of *A. diaparrima* and *A. leiocalyx* leaf extract to inhibit the growth of some bacterial triggers of autoimmune inflammatory diseases, both alone and in combination with conventional antibiotics. The methanolic (and to a lesser extent, the aqueous) *Acacia* spp. extracts were identified as effective growth inhibitors of *P. mirabilis, K. pneumoniae* and *A. baylyi*, but were ineffective against *P. aeruginosa*. *Proteus mirabilis* was particularly susceptible to inhibition (MIC values of 110 and 378µg/mL for the methanolic and aqueous extracts respectively). Whilst the methanolic and aqueous *Acacia* spp. extracts were substantially less potent inhibitors of *K. pneumoniae* and *A. baylyi* growth, the MIC values recorded against these bacteria was generally <1000µg/mL. Thus, these extracts and thus may be useful for preventing and treating rheumatoid arthritis, ankylosing spondylitis and multiple sclerosis, as well as other diseases caused by these bacteria.

Whilst a detailed investigation of the phytochemistry of the *A. diaparrima* and *A. leiocalyx* leaf extracts was beyond the scope of this study, the qualitative phytochemical studies highlighted several phytochemical classes that may contribute to the bacterial growth inhibitory activity. The methanolic and aqueous extracts had relatively high abundances in polyphenolics, flavonoids and tannins. Many studies have reported potent antibacterial activities for a wide variety of flavonoids.[36,37] This has been attributed to a variety of mechanisms, including their ability to complex with extracellular and soluble proteins and as well as bacterial cell walls.[37] Similarly, multiple tannins have broad spectrum antibacterial activity via a variety of intra- and extracellular mechanisms, including the precipitation of microbial proteins.[38,39] Phenolics are toxic to microorganisms via enzyme inhibition mechanisms, possibly through non-specific interaction with proteins or by reaction with sulfhydryl groups.[39,40] It is also

likely that other phytochemical classes may also contribute to the growth inhibitory properties of these extracts. Therefore, phytochemical evaluation studies and bioactivity driven isolation of active components are required to evaluate the mechanism of the *diaparrima* and *A. leiocalyx* leaf extracts growth inhibition.

The combinational studies with conventional antibiotics were perhaps of greater interest. Several combinations displayed substantially greater potential as therapeutic agents against bacterial triggers of rheumatoid arthritis (*P. mirabilis*), ankylosing spondylitis (*K. pneumoniae*) and multiple sclerosis (*A. baylyi*) than either the extracts or antibiotics did alone. Three synergistic combinations were identified in this study (two against *P. mirabilis*, one against *A. baylyi*). Notably, all of these combinations contained tetracycline as the conventional antibiotic component. The implications of a synergistic interaction include enhanced efficacy, thereby allowing lower dose administration, with reduced side effects and possibly reduced antimicrobial resistance, or conversely greater efficacy with administration of the same dosage.[8] Notably *A. baylyi*, was initially resistant to tetracycline. Thus, this study identified combinations of plant extracts and antibiotics which may repurpose tetracycline and greatly enhance its efficacy, even against otherwise resistant bacterial strains. Interestingly, all of the synergistic combinations contained methanolic *Acacia* spp. leaf extracts as the extract component, suggesting the presence of a common active compound or class of compounds that may be responsible for the synergistic effects. Furthermore, all of the synergistic combinations produced a relatively wider range of interactions, including synergistic and additive interactions at different ratios. This is consistent with reversible competition between the extract component(s) and the conventional antibiotic for binding to an effector.[8,41]

Microbes have developed numerous resistance mechanisms to avoid the effects of antibiotics. One main method is through the use of Multi-Drug Resistant (MDR) efflux pumps which are encoded chromosomally and utilized to rapidly remove antibiotics that have entered the bacterial cells, thus rendering them resistant to the effects of the antibiotic.[8] A single pump may allow the bacteria to escape several types of antimicrobials. When these efflux pumps are inhibited, the intracellular concentration of antibiotic will increase, allowing the treatment to once again be effective. Interestingly, many plants possess MDR pump inhibitors in order to enhance the activity of their own natural antimicrobial compounds. Such MDR pump inhibitors become great tools when used in combination with some previously ineffective/resistance prone antibiotic compounds.[8] Surprisingly, there are currently no EPI/antimicrobial drug combinations on the market and much more research is needed in this area.

Synergy was only detected in this study in combinations containing tetracycline. Notably, efflux pumps are the main bacterial resistance mechanism which renders tetracycline inactive.[42,43] A total of nine multidrug efflux systems have been identified including Tet (A), a potent tetracycline efflux protein.[42] It is therefore possible that *A. diaparrima* and *A. leiocalyx* leaf extract component(s) may be inhibiting one or more tetracycline efflux pumps, thereby blocking tetracycline efflux from the cell and allowing the antibiotic to inhibit bacterial protein synthesis. However, further studies are required to confirm this.

The preparation and usage of combinations of *A. diaparrima* and *A. leiocalyx* leaf extract/compound with conventional antibiotic will depend on the nature of the pathogen and of the disease treated. In general, combinations of antibiotic with pure *A. diaparrima* and *A. leiocalyx* leaf derived compounds would be preferred for acute infections as they are much less complex, easier to standardize and have lower chances of unwanted side effects. The use of crude extracts in these preparations is also effective and may still be acceptable to treat some diseases. However, when treating chronic illness, or using a combinational approach to prevent illness (as would be required in preventing autoimmune inflammatory diseases), the use of a pure potentiator compound in combination with the antibiotic may not be desirable. Continuous exposure of bacteria to a pure antibiotic (or to a combination of a single antibiotic and single potentiator) is likely to induce resistance to one or both of the compounds in the bacteria. Indeed, some *E. coli* strains are now resistant to amoxicillin-clavulanic acid combinations.[8] However, crude plant extracts often contain numerous antibacterial compounds which may affect multiple bacterial targets. Thus, using a plant extract (rather than pure plant compounds) in combination with an antibiotic is less likely to result in resistant bacteria. Indeed, we were unable to find any reports of any bacteria developing resistance to a crude plant extract. For this reason, lowest extract: highest antibiotic ratio which produced synergy may be the preferred ratio for treating acute bacterial infections, whilst the highest extract: lowest antibiotic ratio which produced synergy may be preferred for preventing and treating chronic autoimmune diseases.

None of the *A. diaparrima* and *A. leiocalyx* leaf extracts or conventional antibiotics, displayed toxicity in either the ALA toxicity assay. Similarly, all combinations were nontoxic in the ALA assay, confirming their potential for therapeutic use. The non-toxicity of the conventional antibiotics is hardly surprising as these drugs have a long history of therapeutic use and their lack of toxicity has previously been verified in clinical trials. The lack of toxicity determined for the *A. diaparrima* and *A. leiocalyx* leaf extracts may perhaps also be unsurprising as *Acacia* spp. have been used by Australian Aborigines therapeutically for perhaps thousands of years.[44] However, there is a relative lack of prior reports of rigorous toxicity studies for *A. diaparrima* and *A. leiocalyx* leaf extracts. Whilst the lack of toxicity detected for the combinations indicate their potential for therapeutic usage, further *in vitro* studies using other human cell lines are required

to verify their safety. Furthermore, *in vivo* testing is also required to confirm that the extracts and combinations retain efficacy and remain nontoxic in complex biological systems.

CONCLUSION

The majority of the conventional antibiotic and *A. diaparrima* and *A. leiocalyx* leaf extract combinations demonstrated additive or indifferent interactions. Whilst these combinations may have limited added benefit compared with using the conventional antibiotic (or extract) alone, they do alleviate some concerns related to concurrent use of the two forms of healthcare as these interactions indicate that neither therapy is reducing the efficacy of the other therapy. Synergy was seen for three of the antibiotics: medicinal plant combinations studied. The implications of these synergistic interaction include enhanced efficacy, thereby allowing lower doses to be administered, thus reducing any side effects of the chemotherapy. Of further benefit, bacterial exposure to lower levels of the conventional antibiotics is likely to decrease the induction of further antibiotic resistance. Whilst the findings reported here indicate the potential of *A. diaparrima* and *A. leiocalyx* leaf extracts (particularly in combination with tetracycline) as preventative and therapeutic options against bacterial triggers of some autoimmune inflammatory diseases, further *in vivo* investigations are required to support these *in vitro* findings. Furthermore, studies to determine the possible mechanism of action resulting in the observed interaction are warranted, and bioactivity driven compound isolation and/or metabolomics studies are also required to determine the active compound(s), as well as those responsible for the antibiotic potentiation, within the *A. diaparrima* and *A. leiocalyx* leaves.

ACKNOWLEDGEMENT

The financial assistance of the Environmental Futures Research Institute and the School of Environment and Science, Griffith University is hereby acknowledged.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ALA: Brine-shrimp lethality assay; **DMSO:** Dimethyl sulfoxide; **HDF:** Human dermal fibroblasts; **INT:** ρ-iodonitrotetrazolium chloride; **LD_{50}:** Dose of sample necessary to have a lethal effect on 50% of test organisms or cells; **MIC:** Minimum inhibitory concentration; **ΣFIC:** The sum of the fractional inhibitory concentration.

SUMMARY

- *Acacia diaparrima* and *Acacia leicalyx* leaf extracts inhibit the growth of several bacterial triggers of selected autoimmune diseases.
- The methanolic and aqueous extracts were good growth inhibitors against all bacteria except *P. aeruginosa*.
- Several combinations of the *Acacia* spp. extracts and tetracycline had greater activity against *P. mirabilis* than the extract or antibiotic components individually.
- All extracts were nontoxic in the *Artemia* nauplii toxicity assay.

REFERENCES

1. Cock IE, Cheesman MJ. The early stages of multiple sclerosis: new targets for the development of combinational drug therapies. In: Neurological disorders and imaging physics. Application of Multiple Sclerosis. 2019;1. doi: 10.1088/978-0-7503-1762-7ch2.
2. Cock IE, Cheesman MJ. The potential of plants of the genus *Syzygium* (Myrtaceae) for the prevention and treatment of arthritic and autoimmune diseases. In: Bioactive Foods as Dietary Interventions for Arthritis, osteoarthritis, and related Autoimmune Diseases. 2nd ed. Editors Preedy VR, Watson RR. Elsevier Publishing. 2018.
3. Aletaha D, Kapral T, Smolen JS. Toxicity profiles of traditional disease modifying antirheumatic drugs for rheumatoid arthritis. Ann Rheum Dis. 2003;62(5):482-6. doi: 10.1136/ard.62.5.482, PMID 12695166.
4. Ebringer A, Rashid T. Rheumatoid arthritis is an autoimmune disease triggered by *Proteus* urinary tract infection. Clin Dev Immunol. 2006;13(1):41-8. doi: 10.1080/17402520600576578.
5. Ebringer A, Cunningham P, Ahmadi K, Wrigglesworth J, Hosseini R, Wilson C. 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. doi: 10.1136/ard.51.11.1245.
6. Hughes LE, Smith PA, Bonell S, Natt RS, Wilson C, Rashid T, *et al*. Cross-reactivity between related sequences found in *Acinetobacter* sp., *Pseudomonas aeruginosa*, myelin basic protein and myelin oligodendrocyte glycoprotein in multiple sclerosis. J Neuroimmunol. 2003;144(1-2):105-15. doi: 10.1016/s0165-5728(03)00274-1, PMID 14597104.
7. Carapetis JR, McDonald M, Wilson NJ. Acute rheumatic fever. Lancet. 2005;366(9480):155-68. doi: 10.1016/S0140-6736(05)66874-2, PMID 16005340.
8. Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing new antimicrobial therapies: are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution? Pharmacogn Rev. 2017;11(22):57-72. doi: 10.4103/phrev.phrev_21_17, PMID 28989242.
9. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, *et al*. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist. 2018;11:1645-58. doi: 10.2147/IDR.S173867, PMID 30349322.
10. Gross M. Antibiotics in crisis. Curr Biol. 2013;23(24):R1063-5. doi: 10.1016/j.cub.2013.11.057, PMID 24501765.
11. WHO. The evolving threat of antimicrobial resistance: options for action. World Health Organization; 2014 [cited Mar 14 2017]. Available from: http://apps.who.int/iris/bitstream/10665/44812/1/9789241503181_eng.pdf.
12. Van Vuuren S, Viljoen A. Plant-based antimicrobial studies–methods and approaches to study the interaction between natural products. Planta Med. 2011;77(11):1168-82. doi: 10.1055/s-0030-1250736, PMID 21283954.
13. Abreu AC, McBain AJ, Simões M. Plants as sources of new antimicrobials and resistance-modifying agents. Nat Prod Rep. 2012;29(9):1007-21. doi: 10.1039/c2np20035j, PMID 22786554.
14. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008;15(8):639-52. doi: 10.1016/j.phymed.2008.06.008, PMID 18599280.
15. Cottarel G, Wierzbowski J. Combination drugs, an emerging option for antibacterial therapy. Trends Biotechnol. 2007;25(12):547-55. doi: 10.1016/j.tibtech.2007.09.004, PMID 17997179.
16. Ali M. Antimicrobial metabolites from Australian Acacia [PhD thesis]. University of Western Australia. 1998.
17. Arias ME, Gomez JD, Cudmani NM, Vattuone MA, Isla MI. Antibacterial activity of ethanolic and aqueous extracts of *Acacia aroma* Gill. ex Hook et Arn. Life Sci. 2004;75(2):191-202. doi: 10.1016/j.lfs.2003.12.007, PMID 15120571.

18. Seigler DS. Phytochemistry of *Acacia sensu-lato*. Biochem Syst Ecol. 2003;31(8):845-73. doi: 10.1016/S0305-1978(03)00082-6.
19. Hegarty MP, Hegarty EE. Food safety of Australian bushfoods, publication no. 01/28. Australia: Rural Industries Research and Development Corporation; 2001.
20. Latz PK. Bushfires and bushtucker: aboriginal plant use in Central Australia. Alice Springs, NT, Australia: IAD Press; 1995.
21. Maen A, Cock IE. Inhibitory activity of Australia culinary herb extracts against the bacterial triggers of selected autoimmune diseases. Pharmacogn Commun. 2015;5(2):130-9. doi: 10.5530/pc.2015.2.4.
22. Kalotas A, Goddard C. Punu, Yankunytjatjara plant use. Alice Springs, NT, Australia: Institute for Aboriginal Development; 1985.
23. Cock IE. Medicinal and aromatic plants – Australia. Available from: http://www.eolss.net. In: Ethnopharmacology section, Biological, Physiological and Health Sciences, Encyclopedia of Life Support Systems (EOLSS). Oxford, UK: EOLSS Publishers; 2011; Developed under the Auspices of the UNESCO.
24. Cock IE. Australian *Acacia* spp. extracts as natural food preservatives: growth inhibition of food spoilage and food poisoning bacteria. Pharmacogn Commun. 2017;7(1):4-15.
25. Wright MH, Lee CJ, Pollock CE, *et al*. Growth inhibitory activity of selected high antioxidant Australian *Syzygium* species against the food poisoning and tissue necrotic pathogen *Clostridium perfringens*. Pharmacogn Commun. 2016;6(2):93-9. doi: 10.5530/pc.2016.2.7.
26. Nel AL, Murhekar S, Matthews B, *et al*. The interactive antimicrobial activity of *Terminalia sericea* Burch. ex DC. leaf extracts and conventional antibiotics against bacterial triggers of selected autoimmune inflammatory diseases. S Afr J Bot. 2020;133:17-29.
27. Courtney R, Sirdaarta J, Matthews B, Cock IE. Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbial triggers of autoimmune inflammatory diseases. Pharmacogn J. 2015;7(1):18-31. doi: 10.5530/pj.2015.7.2.
28. Hutchings A, Cock IE. The interactive antimicrobial activity of *Embelica officinalis* Gaertn. fruit extracts and conventional antibiotics against some bacterial triggers of autoimmune inflammatory diseases. Pharmacogn J. 2018;10(4):654-62. doi: 10.5530/pj.2018.4.108.
29. Ilanko P, McDonnell PA, Van Vuuren SF, *et al*. Interactive antibacterial profile of *Moringa oleifera* Lam. extracts and conventional antibiotics against bacterial triggers of some autoimmune inflammatory diseases. S Arf. J Bot. 2019;124:420-35.
30. Hübsch Z, Van Zyl RL, Cock IE, Van Vuuren SF. Interactive antimicrobial and toxicity profiles of conventional antimicrobials with southern African medicinal plants. S Afr J Bot. 2014;93:185-97. doi: 10.1016/j.sajb.2014.04.005.
31. Ilanko A, Cock IE. The interactive antimicrobial activity of conventional antibiotics and *Petalostigma* spp. extracts against bacterial triggers of some autoimmune inflammatory diseases. Pharmacogn J. 2019;11(2):292-309. doi: 10.5530/pj.2019.11.45.
32. Winnett V, Sirdaarta J, White A, Clarke FM, Cock IE. Inhibition of *Klebsiella pneumoniae* growth by selected Australian plants: natural approaches for the prevention and management of ankylosing spondylitis. Inflammopharmacology. 2017;25(2):223-35. doi: 10.1007/s10787-017-0328-1, PMID 28239782.
33. Wang Y, Liang Y, Cock IE. *Rosa canina* L. fruit extracts inhibit the growth of bacterial triggers of some autoimmune inflammatory diseases and potentiate the activity of conventional antibiotics. Pharmacogn Commun. 2020;10(1):7-17.
34. Cock IE, Kalt FR. Toxicity evaluation of *Xanthorrhoea johnsonii* leaf methanolic extract using the *Artemia franciscana* bioassay. Pharmacogn Mag. 2010;6(23):166-71. doi: 10.4103/0973-1296.66929, PMID 20931073.
35. Ruebhart DR, Wickramasinghe W, Cock IE. Protective efficacy of the antioxidants vitamin E and trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* nauplii. J Toxicol Environ Health A. 2009;72(24):1567-75. doi: 10.1080/15287390903232459, PMID 20077231.
36. Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J Pharmacol. 2001;33(1):2-16.
37. Cock IE. The phytochemistry and chemotherapeutic potential of *Tasmannia lanceolata* (Tasmanian pepper): a review. Pharmacogn Commun. 2013;3(4):1-13.
38. Buzzini P, Arapitsas P, Goretti M, Branda E, Turchetti B, Pinelli P, *et al*. Antimicrobial activity of hydrolysable tannins. Mini Rev Med Chem. 2008;8(12):1179-87. doi: 10.2174/138955708786140990, PMID 18855732.
39. Cock IE. The medicinal properties and phytochemistry of plants of the genus *Terminalia* (Combretaceae). Inflammopharmacology. 2015;23(5):203-29. doi: 10.1007/s10787-015-0246-z, PMID 26226895.
40. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-82. doi: 10.1128/CMR.12.4.564, PMID 10515903.
41. Ramón-Garcia S, González del Rio R, Villarejo AS, Sweet GD, Cunningham F, Barros D, *et al*. Repurposing clinically approved cephalosporins for tuberculous therapy. Sci Rep. 2016;6:34239.
42. Mikulášová M, Chovanová R, Vaverková Š. Synergism between antibiotics and plant extracts or essential oils with efflux pump inhibitory activity in coping with multidrug-resistant *staphylococci*. Phytochem Rev. 2016:1-35.
43. Kim TH, Raiz A, Unni AD, Murhekar S, Donose BC, Floetenmeyer M, *et al*. Combating antibiotic-resistant gram-negative bacteria strains with tetracycline-conjugated carbon nanoparticles. Adv Biosyst. 2020;4(9):e2000074. doi: 10.1002/adbi.202000074, PMID 32803868.
44. Cock IE. Medicinal and aromatic plants – Australia [cited Apr 1 2013]. Available from: http://www.eolss.net. In: Ethnopharmacology, Encyclopedia of Life Support Systems (EOLSS), 2011. Developed under the auspices of UNESCO. Oxford, UK: EOLSS Publishers; 2011.

Cite this article: Guo R, Yang X, Cock IE. Antimicrobial Activity of *Acacia disparrima* Benth. and *Acacia leiocalyx* Pedley Leaf Extracts in Combination with Antibiotics against Bacterial Triggers of Selected Autoimmune Diseases. Pharmacognosy Communications. 2023;13(3):104-15.