Pharmacogn. Commn. 2022;12(2):65-73.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

Curcumin, Lupeol and Piperine Inhibit the Bacterial Triggers of some Autoimmune Inflammatory Diseases and Potentiate the Activity of Conventional Antibiotics

Audric Puisais[1], Ian E Cock[1,2,*]
[1]School of Environment and Science, Griffith University, Nathan, Queensland, AUSTRALIA.
[2]Centre for Planetary Health and Food Security, Nathan Campus, Griffith University, Nathan, Queensland, AUSTRALIA.

ABSTRACT

Background: Curcumin, lupeol and piperine are phytochemical constituents of multiple plants that are used traditionally to treat inflammation, including for treating autoimmune inflammatory diseases. Despite this, relatively few studies have examined the ability of these compounds to inhibit the etiological events of these diseases by examining their ability to inhibit their bacterial triggers. **Methods:** The ability of curcumin, lupeol and piperine to inhibit the growth of some bacterial triggers of selected autoimmune inflammatory diseases was screened using disc diffusion assays and quantified by liquid dilution MIC assays. The ΣFIC of the pure plant compound/conventional antibiotic combinations was determined and used to classify the class of interaction. Toxicity was evaluated using the *Artemia* nauplii cytotoxicity assay. **Results:** Curcumin, lupeol and piperine strongly inhibited the growth of several bacterial triggers of autoimmune diseases, with MIC values as low as 178 µg/mL against some bacteria. Furthermore, combining these compounds with conventional antibiotics resulted in significant potentiation of the inhibitory activity for some combinations, with one synergistic and nineteen additive combinations detected. Notably, three antagonistic combinations were also noted. None of the individual components (nor the combinations) were significantly toxic in the ALA toxicity assay. **Conclusion:** Curcumin, lupeol and piperine inhibit the growth of some bacterial triggers of rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis and rheumatic fever. Additionally, these compounds substantially potentiated the effects of some conventional antibiotics against these bacteria. Further *in vivo* studies to determine the anti-inflammatory and antibacterial mechanisms are warranted.

Key words: Curcumin, Lupeol, Piperine, Antibacterial activity, Synergy, Rheumatoid arthritis, Ankylosing spondylitis, Multiple sclerosis.

Correspondence:
Dr. I E Cock
[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.
E-mail: i.cock@griffith.edu.au
DOI: 10.5530/pc.2022.2.14

INTRODUCTION

There are currently no effective widely available cures for the autoimmune inflammatory diseases rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis and rheumatic fever.[1,2] Instead, the current treatment modality aims to alleviate the symptoms of these diseases by administering anti-inflammatory drugs (particularly non-steroidal anti-inflammatory drugs (NSAIDs)) and analgesics. Whilst this approach increases the patient's comfort whilst their immune system overcomes the cause of the illness, it does not significantly alter the progression of the disease, nor does it mitigate autoimmune damage to self-tissue. Furthermore, prolonged anti-inflammatory and/or analgesic drug usage induces toxicity and is associated with numerous negative side effects.[3] For example, prolonged usage of cyclooxygenase-2 (COX-2) inhibitory NSAIDs increases the risk of myocardial infarction.[4] Novel therapeutic chemotherapeutics that not only down-regulate the inflammatory related symptoms of the disease, but also target their etiology, may be substantially more effective in treating these diseases and may allow for prevention through prophylactic usage. This may protect genetically susceptible people from tissue damage, as well as substantially decreasing the later phase inflammatory symptoms of autoimmune diseases.

Autoimmune inflammatory diseases occur when exposure of the host's immune system to specific antigens stimulates the production of self-reactive antibodies.[1,2,5] Some autoimmune inflammatory diseases may be triggered by environmental and dietary stimuli,[1] although others are triggered by bacterial pathogens. Targeting these autoimmune antigenic triggers in genetically susceptible people may block the etiology of the disease, as well as preventing the downstream immunological and inflamatory events of thes diseases. Interestingly, many of the antigenic triggers of autoimmune inflammatory diseases have been identified via serotyping and genotyping studies, allowing for the the development of novel chemotherapies targeting the diseases' etiology.[1] It is well established that *Proteus mirabilis* can trigger rheumatoid arthritis in genetically susceptible people,[6] *Klebsiella pneumoniae* may trigger ankylosing spondylitis,[7] *Acinetobacter baylyi* and *Pseudomonas aeruginosa* infections can trigger multiple sclerosis in genetically susceptible people,[8] and *Streptococcus pyogenes* can induce rheumatic fever.[9]

An examination of plant-based therapies and traditional medicines may highlight promising leads for the treatment of inflammation and autoimmune inflammatory diseases as plants have long been used to treat both inflammation and pathogenic diseases.[1,10] Furthermore, previous research has already identified and characterised multiple phytochemicals with therapeutic properties relevant to to treatment of autoimmune diseases. Whilst multiple molecular targets have been identified, several compounds are particularly interesting due to their ability to which target different aspects of complex inflammatory diseases via different mechanisms. Curcumin (Figure 1a) is isolated from *Curcuma longa* L. rhizome and is commonly known as turmeric. It is particularly promising for the treatment of autoimmune diseases as it modulates multiple cellular processes relevant to human health. The anti-inflammatory activity of curcumin (and turmeric) has been extensively reported and is summarised in detail elsewhere.[11] In particular, curcumin can decrease cellular oxidative stress via scavenging reactive oxygen (ROS) and reactive nitrogen species (RNS),[12] as well as modulating the activity of GSH, catalase and superoxide dismutase (SOD).[13,14] Curcumin also inhibits TNF-α stimulation of NF-κB activity.[15] It also inhibits multiple other pro-inflammatory mechanisms.[11] Additionally, curcumin

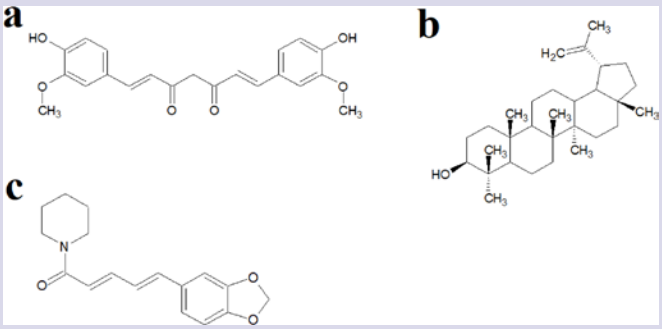


Figure 1: Structures of (a) curcumin (keto form), (b) lupeol, and (c) piperine.

(and turmeric) has significant inhibitory activity against multiple bacterial pathogens.[16] Aqueous turmeric rhizome extracts have strong antibacterial activity (MIC 16-128 µg/mL) against multiple bacteria including *Klebsiella pneumoniae*.[17-19] Similarly, pure curcumin inhibits the growth of multiple bacteria, including *Pseudomonas aeruginosa*[20] and methicillin-resistant *Staphylococcus aureus* (MRSA), with MICs 125-250 µg/mL.[21] However, the ability of curcumin and turmeric to inhibit the growth of many other bacteria (including many bacterial triggers of autoimmune diseases) is yet to be verified. Furthermore, few studies have examined the potential of curcumin and turmeric to potentiate the activity of conventional antibiotics.

The pentacyclic triterpenoid lupeol (Figure 1b) also has potential for the treatment of autoimmune inflammatory diseases.[22] Lupeol administration significantly decreases prostaglandin E2 (PGE2) production in A23187-stimulated macrophages via inhibition of cyclooxygenase activity.[23,24] Lupeol suppresses macrophage phagocytosis activity and suppresses T cell mediated cytokine production,[25] as well as decreasing the production of the pro-inflammatory cytokines TNF-α and IL-β in stimulated macrophages.[23] It also inhibits the release of IL-4, IL-5 and IL-13 in an asthmatic mouse model.[26] Other studies have screened lupeol for antibacterial activity, although there are significant differences between the different studies. Lupeol has been reported to inhibit the growth of *Escherichia coli, P. aeruginosa* and *S. aureus*, with MICs of 250 µg/mL.[27] Another study reported that *P. aeruginosa* and *K. pneumoniae* are susceptible to lupeol.[28] However, that study only tested single, relatively high concentrations of lupeol. MIC values were not determined, making comparisons with other studies impossible. In contrast, multiple other studies have reported that lupeol was ineffective against other bacterial pathogens including *Mycobacterium tuberculosis*.[29] Substantial further work is required to confirm and quantify the antibacterial properties of lupeol.

Multiple studies have also highlighted the therapeutic properties of piperine (Figure 1c; a component of *Piper nigrum* L.) and these have been reviewed extensively elsewhere.[30] Zhai *et al.* examined its anti-inflammatory properties and reported that piperine inhibits the production of the transcription factor NF-κB, thereby modulating the release of numerous cytokines.[31] Indeed, significant reductions in the release of the pro-inflammatory cytokines TNF-α, IL-1β[31,32] and IL-6, as well as increases in anti-inflammatory cytokines, including IL-10 were noted.[31] Additionally, piperine also inhibits cellular signalling pathways via inhibiting MAPK, ERK, and JNK, as well as reducing the expression of toll-like receptor proteins TLR-2 and TLR-4.[31] Therefore, piperine may be useful in alleviating the downstream inflammatory symptoms of the autoimmune inflammatory diseases. Furthermore, piperine may inhibit the etiology of some autoimmune diseases by inhibiting their bacterial triggers. Indeed, several studies have reported substantial bacterial growth inhibitory activity for piperine against a variety of bacterial pathogens. Piperine induces proliferation of B and T cells and enhances macrophage activation in mice exposed to *M. tuberculosis*.[33] Piperine also directly inhibits the growth of some bacteria, including *Bacillus cereus, Enterococcus faecalis, Staphylococcus zylosus, Staphylococcus arreus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia* and *Salmonella enterica*.[34] Despite these earlier studies, the growth inhibitory properties of curcumin, lupeol and piperine have not been extensively tested against the bacterial triggers of rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis or rheumatic fever. This study aimed to address this gap in the literature by quantifying the antibacterial activity of these compounds against *Proteus mirabilis. Klebsiella pneumoniae, Acinitobacter baylyi, Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Furthermore, this study also examines the effects of curcumin, lupeol and piperine in combination with some conventional antibiotics against those bacteria.

MATERIALS AND METHODS

Plant test compounds

Curcumin, lupeol and piperine (AR grade) were purchased from Sigma, Australia and dissolved in 5% DMSO (AR grade; Chem-Supply, Australia) to give a stock concentration of 1 mg/mL and stored as aliquots at -30°C.

Antibacterial analysis

Conventional Antibiotics

Chloramphenicol (≥98 % purity by HPLC), ciprofloxacin ((≥98 % purity by HPLC), erythromycin (potency ≥850 µg/mg), gentamicin (potency of 600 µg/mg), penicillin-G (potency of 1440-1680 µg/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. Standard discs of ampicillin (10 µg) and chloramphenicol (10 µg) were obtained from Oxoid Ltd., Australia and used as positive controls in the disc diffusion susceptibility assays.

Bacterial cultures

All of the bacterial strains tested in this study were included because they can trigger autoimmune inflammatory diseases in genetically susceptible individuals.[1,2] Reference strains of *Proteus mirabilis* (ATCC21721), *Klebsiella pneumoniae* (ATCC31488), *Acinetobacter baylyi* (ATCC33304), *Pseudomonas aeruginosa* (ATCC39324) and *Streptococcus pyogenes* (ATCC12384) were purchased from American Type Culture Collection, USA. 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 sub-cultured and maintained in nutrient broth at 4°C until use.

Evaluation of bacterial susceptibility to growth inhibition

The susceptibility of the bacteria to the plant compounds and the conventional antibiotics was assessed undiluted using modified disc diffusion assays.[35] Ampicillin (10 µg) and chloramphenicol discs (10 µg) 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 antibacterial activity of the plant compounds and the conventional antimicrobials (both independently and in combinations) was quantified

using standard liquid dilution MIC assays.[36] Briefly, to test the individual compounds separately, 100 µL of sterilized distilled water was dispensed into each well of 96 well micro-titre plate. The plant compounds or the conventional antibiotics (100 µL) were then dispensed into separate wells of the first row of the plate. Nutrient broth (negative control), a sterile control (media without bacteria) and a sample-free culture control (to ensure the media was capable of supporting microbial growth) were also included on all plates to verify correct assay function. Following sample addition, each test was serially diluted down each column of the plate using doubling serial dilution. A 100 µL volume of bacterial culture (containing approximately $1x10^6$ colony forming units (CFU)/mL) was added to all wells of the plate (excluding the sterile control wells). All plates were then incubated at 37°C for 24 hr. p-Iodonitrotetrazolium violet (INT) (Sigma-Aldrich, Australia) was dissolved in sterile deionised water to prepare a 0.2 mg/mL INT stock solution. A 40 µL volume of this stock solution was added into all wells and the plates were incubated for 6 hr at 30°C to allow full colour development. The MIC was visually determined as the lowest dose at which colour development was completely inhibited.

Extract-conventional antibiotic interaction studies: ΣFIC assessment

The effect of the plant compounds in combination with the conventional antibiotics was further classified by sum of the fractional inhibitory concentration (ΣFIC) analysis. The FIC was calculated using the following equation, where (a) represents the conventional antibiotic and (b) represents the plant compound:[37,38]

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

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

The ΣFIC was calculated by: $\Sigma FIC = FIC^{(i)} + FIC^{(ii)}$. The combinational effects were classified as synergistic for ΣFIC values of ≤ 0.5, additive (> 0.5 – 1.0), indifferent (> 1.0 – ≤ 4.0) or antagonistic (> 4.0).[38,39]

Toxicity screening

The toxicity of the plant compounds, conventional antibiotics and reference toxin were assessed using a standardised *Artemia franciscana* nauplii lethality assay (ALA).[39] 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 following 24 hr exposure and is espressed 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 least three independent experiments, each with internal triplicates (n=9). 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

Bacterial growth inhibition screening

Proteus mirabilis growth was particularly susceptible to curcumin, lupeol and piperine, with ZOIs of 8-8.4 mm (Figure 2). The inhibition by these compounds in noteworthy as it is similar to the ZOIs produced by ampicillin and tetracycline (8 and 8.2 mm respectively). In contrast,

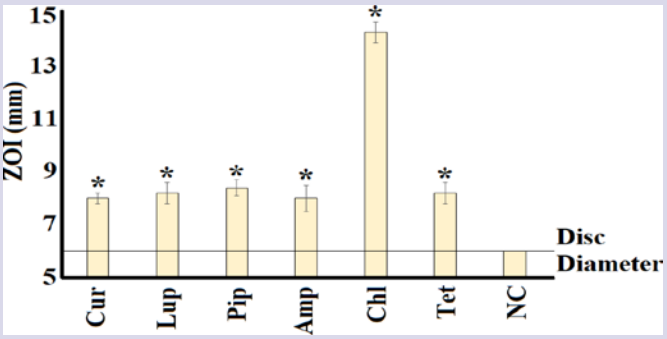


Figure 2: Antibacterial activity of the pure compounds against *P. mirabilis* (ATCC21721) measured as zones of inhibition (mm). Cur = curcumin; Lup = lupeol; Pip = piperine; Amp = ampicillin (10 µg); Chl = chloramphenicol (10 µg); Tet = tetracycline (10 µg); *NC = negative control (nutrient broth)*. Results are expressed as mean zones of inhibition of three replicates, each with internal triplicated (n=9)± SEM * indicates results that are significantly different to the negative control (P<0.01).

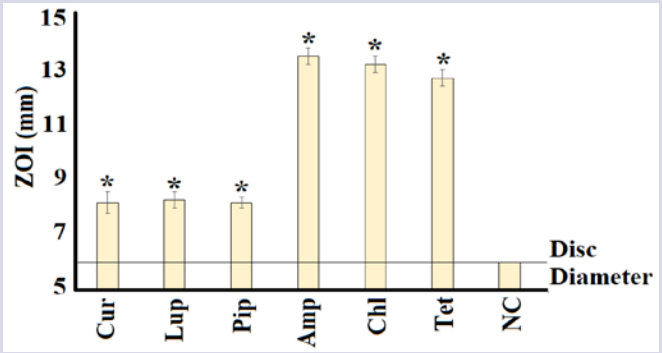


Figure 3: Antibacterial activity of the pure compounds against *K. pneumoniae* (ATCC31488) measured as zones of inhibition (mm). Cur = curcumin; Lup = lupeol; Pip = piperine; Amp = ampicillin (10 µg); Chl = chloramphenicol (10 µg); Tet = tetracycline (10 µg); NC = negative control (nutrient broth). Results are expressed as mean zones of inhibition of three replicates, each with internal triplicated (n=9)± SEM * indicates results that are significantly different to the negative control (P<0.01).

the *P. mirabilis* strain tested in this study was highly susceptible to chloramphenicol, with a ZOI of 14.3 mm. The pure plant compounds also strongly inhibited the growth of *K. pneumoniae*, with ZOIs of approximately 8.2 mm (Figure 3) The notable growth inhibitory activity of the pure plant compounds against *P. mirabilis* (a bacterial trigger of rheumatoid arthritis) and *K. pneumoniae* (a trigger of ankylosing spondylitis) indicates that they have potential for use in the prevention and treatment of these diseases, as well as other diseases caused by these pathogens.

All of the pure plant compounds also inhibited of the growth of *A. baylyi* (a bacterial trigger of multiple sclerosis in genetically susceptible people), albeit with substantially smaller ZOIs (6.7, 6.4 and 7 mm for curcumin, lupeol and piperine respectively) (Figure 4). This bacterial strain was also found to be relatively resistant to the ampicillin control (ZOI = 7.8 mm), although the chloramphenicol and tetracycline controls produced relatively large ZOIs (9.2 and 10.4 mm respectively). The pure plant compounds also inhibited the growth of another bacterial trigger of multiple sclerosis, *P. aeruginosa* (Figure 5), albeit with small ZOIs

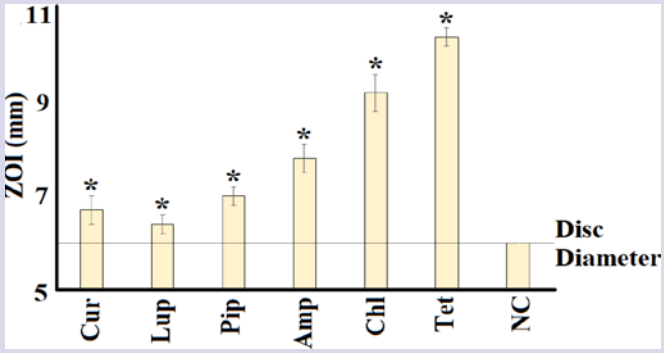


Figure 4: Antibacterial activity of the pure compounds against *A. baylyi* (ATCC33304) measured as zones of inhibition (mm). Cur = curcumin; Lup = lupeol; Pip = piperine; Amp = ampicillin (10 µg); Chl = chloramphenicol (10 µg); Tet = tetracycline (10 µg); NC = negative control (nutrient broth). Results are expressed as mean zones of inhibition of three replicates, each with internal triplicated (*n*=9)± SEM * indicates results that are significantly different to the negative control (*P*<0.01).

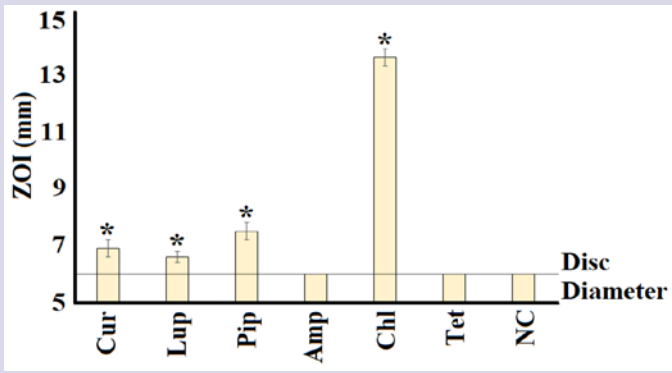


Figure 5: Antibacterial activity of the pure compounds against *P. aeruginosa* (ATCC: 39324) measured as zones of inhibition (mm). Cur = curcumin; Lup = lupeol; Pip = piperine; Amp = ampicillin (10 µg); Chl = chloramphenicol (10 µg); Tet = tetracycline (10 µg); NC = negative control (nutrient broth). Results are expressed as mean zones of inhibition of three replicates, each with internal triplicated (*n*=9)± SEM * indicates results that are significantly different to the negative control (*P*<0.01).

(6.9, 6.6 and 7.5 mm for curcumin, lupeol and piperine respectively). However, despite the relatively small ZOIs, this inhibition is still noteworthy as the *P. aeruginosa* strain tested in this study was completely resistant to both the ampicillin and tetracycline controls (as judged by ZOI), although it was highly susceptible to the chloramphenicol control (13.6 mm). These susceptibilities indicate that curcumin, lupeol and piperine are effective inhibitors of both bacterial triggers of multiple sclerosis.

Curcumin, lupeol and piperine were also moderate inhibitors of *S. pyogenes* growth (Figure 6), with ZOIs of 7.8, 6.9 and 8.6 mm measured respectively. Interestingly, this *S. pyogenes* strain was completely resistant to chloramphenicol, yet susceptible to ampicillin and tetracycline (ZOIs of 8 and 10.4 mm respectively). Thus, curcumin, lupeol and piperine may be useful for preventing and treating rheumatic fever, even against antibiotic resistant *S. pyogenes* strains, as well as treating a range of other illnesses also caused by this *S. pyogenes* infections (including streptococcal pharyngitis and impetigo).

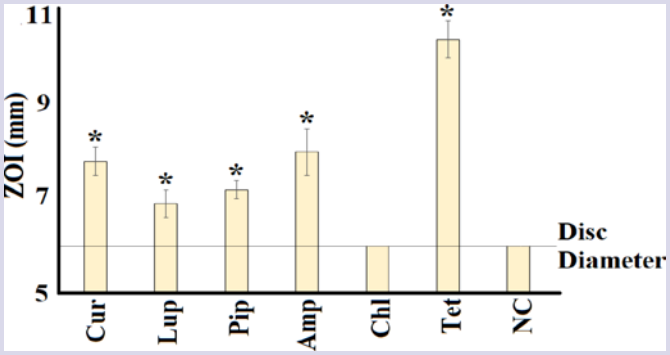


Figure 6: Antibacterial activity of the pure compounds against) *S. pyogenes* (ATCC12384) measured as zones of inhibition (mm). Cur = curcumin; Lup = lupeol; Pip = piperine; Amp = ampicillin (10 µg); Chl = chloramphenicol (10 µg); Tet = tetracycline (10 µg); NC = negative control (nutrient broth). Results are expressed as mean zones of inhibition of three replicates, each with internal triplicated (n=9)± SEM * indicates results that are significantly different to the negative control (*P*<0.01).

Quantification of minimum inhibitory concentration (MIC)

The relative level of antimicrobial activity was further evaluated by determining the MIC values using liquid dilution MIC assays (Table 1). For the pure control antibiotic compounds included in this study, MIC values >1 µg/mL have been defined as indicating resistance,[36-38] and therefore the MICs of the pure antibiotics was determined in µg/mL concentrations. For consistency (and to allow comparison), the concentrations of all pure plant compounds are also reported in µg/mL format in the table. However, we have also provided the MICs as µM concentrations to allow for a more meaningful comparison with the reported activity of other pure compounds in previous studies. Consistent with the antibacterial screening assays, all of the pure plant compounds inhibited the growth of each of the bacteria tested. In general, piperine was the most potent inhibitor of the bacterial strains tested, with MIC values of 178, 268, 356, 356 and 356 µg/mL against *P. mirabilis, K. pneumoniae, A. baylyi, P. aeruginosa* and *S. pyogenes* respectively. Curcumin and lupeol were also good inhibitors of the growth of all of the bacterial strains tested, with similar, albeit slightly lower potency than determined for piperine. The conventional antibiotics also inhibited the growth of all bacteria tested. However, MIC values >1 µg/mL have previously been defined as indicative of resistance in this assay.[36] As the MIC values determined for all bacteria using penicillin, chloramphenicol and erythromycin are >1 µg/mL, all of these bacterial strains were resistant to these antibiotics. Similarly, the *A. baylyi, P. aeruginosa* and *S. pyogenes* strains tested in our study were resistant to tetracycline, whilst *P. mirabilis* and *K. pneumoniae* were tetracycline sensitive. Gentamicin and ciprofloxacin were the most effective antibiotics against these bacteria (as judged by its MIC), with four of the five bacterial pathogens susceptible to each of these antibiotics.

Combinational effects: Fractional inhibitory concentration (FIC) assessment

Of the eighteen pure plant compound and conventional antibiotic combinations tested against *P. mirabilis*, the majority (14 combinations) were non-interactive (Table 2). Whilst no added benefit would be gained from combining these compounds with the conventional antibiotics against *P. mirabilis*, the combinations would be safe to use without compromising the activity of either component. This is important as many patients use conventional and traditional therapies concurrently, often

Table 1: MIC values of the pure compounds expressed in µM and µg/mL units against some microbial triggers of some auto-immune inflammatory diseases.

Bacterial Species	Cur	Lup	Pip	Controls					
				Pen	Chlor	Eryth	Tet	Gent	Cip
P. mirabilis	346 (µg/mL)	266 (µg/mL)	178 (µg/mL)	1.25 (µg/mL)	3.3 (µg/mL)	2.5 (µg/mL)	0.63 (µg/mL)	1.25 (µg/mL)	0.63 (µg/mL)
	940 (µM)	625 (µM)	625 (µM)	3.7 (µM)	10.2 (µM)	3.4 (µM)	1.4 (µM)	2.6 (µM)	1.9 (µM)
K. pneumoniae	346 (µg/mL)	400 (µg/mL)	268 (µg/mL)	2.5 (µg/mL)	2.5 (µg/mL)	1.25 (µg/mL)	0.3 (µg/mL)	0.31 (µg/mL)	0.63 (µg/mL)
	940 (µM)	940 (µM)	940 (µM)	7.5 (µM)	7.7 (µM)	1.7 (µM)	0.7 (µM)	0.6 (µM)	1.9 (µM)
A. baylyi	460 (µg/mL)	533 (µg/mL)	356 (µg/mL)	2.5 (µg/mL)	2.5 (µg/mL)	1.25 (µg/mL)	1.25 (µg/mL)	0.31 (µg/mL)	0.63 (µg/mL)
	1250 (µM)	1250 (µM)	1250 (µM)	7.5 (µM)	7.7 (µM)	1.7 (µM)	2.8 (µM)	0.6 (µM)	1.9 (µM)
P. aeruginosa	699 (µg/mL)	809 (µg/mL)	356 (µg/mL)	1.25 (µg/mL)	1.25 (µg/mL)	1.25 (µg/mL)	1.25 (µg/mL)	0.63 (µg/mL)	1.25 (µg/mL)
	1900 (µM)	1900 (µM)	1250 (µM)	3.7 (µM)	3.9 (µM)	1.7 (µM)	2.8 (µM)	1.2 (µM)	3.8 (µM)
S. pyogenes	346 (µg/mL)	533 (µg/mL)	356 (µg/mL)	3.3 (µg/mL)	2.5 (µg/mL)	3.3 (µg/mL)	2.5 (µg/mL)	0.63 (µg/mL)	0.63 (µg/mL)
	940 (µM)	1250 (µM)	1250 (µM)	9.9 (µM)	7.7 (µM)	4.5 (µM)	5.6 (µM)	1.2 (µM)	1.9 (µM)

Cur = curcumin; Lup = lupeol; Pip = piperine; Pen = penicillin-G; Chlor = chloramphenicol; Eryth = erythromycin; Tet = tetracycline; Gent = gentamycin; Cip = ciprofloxacin. - indicates no inhibition at any dose tested.

Table 2: Combinational effects determined as ∑FIC values for the plant compound - conventional antibiotic combinations, against some bacterial triggers of selected autoimmune inflammatory diseases.

Bacterial Species	Test Compound	Antibiotics					
		Pen	Chlor	Eryth	Tet	Gent	Cip
P. mirabilis	Curcumin	1.64 (IND)	*1 (ADD)*	1.06 (IND)	1.26 (IND)	2.37 (IND)	1.2 (IND)
	Lupeol	1.58 (IND)	*1 (ADD)*	*0.76 (ADD)*	1.56 (ADD)	2.15 (IND)	1.12 (IND)
	Piperine	1.55 (IND)	1.41 (IND)	*0.56 (ADD)*	1.56 (ADD)	1.96 (IND)	1.25 (IND)
K. pneumoniae	Curcumin	2.2 (IND)	1.5 (IND)	4.5 (ANT)	*0.56 (ADD)*	1.38 (IND)	1.42 (IND)
	Lupeol	2.58 (IND)	1.91 (IND)	6.16 (ANT)	*0.83 (ADD)*	1.45 (IND)	1.22 (IND)
	Piperine	2.36 (IND)	1.21 (IND)	3.33 (IND)	*0.54 (ADD)*	1.13 (IND)	*0.71 (ADD)*
A. baylyi	Curcumin	3.22 (IND)	*0.75 (ADD)*	1.13 (IND)	4.12 (ANT)	3.32 (IND)	2.84 (IND)
	Lupeol	2.1 (IND)	*0.71 (ADD)*	*0.65 (ADD)*	1.44 (IND)	3.04 (IND)	2.55 (IND)
	Piperine	2.94 (IND)	1.1 (IND)	2.5 (IND)	2.87 (IND)	2.76 (IND)	2.84 (IND)
P. aeruginosa	**Curcumin**	1.27 (IND)	*0.88 (ADD)*	*0.75 (ADD)*	1.06 (IND)	1.52 (IND)	1.01 (IND)
	Lupeol	1.1 (IND)	1.13 (IND)	1.03 (IND)	1.03 (IND)	1.47 (IND)	*0.71 (ADD)*
	Piperine	1.57 (IND)	*0.63 (ADD)*	1.06 (IND)	1.06 (IND)	1.3 (IND)	1.42 (IND)
S. pyogenes	**Curcumin**	1.7 (IND)	*0.84 (ADD)*	1.18 (IND)	1.02 (IND)	2.16 (IND)	1.83 (IND)
	Lupeol	1.49 (IND)	*0.68 (ADD)*	*0.77 (ADD)*	**0.37 (SYN)**	3.63 (IND)	1.44 (IND)
	Piperine	1.66 (IND)	1.07 (IND)	1.16 (IND)	*0.73 (ADD*	2.48 (IND)	1.88 (IND)

Pen = penicillin-G; Chlor = chloramphenicol; Eryth = erythromycin; Tetracycline = tetracycline; Gent = gentamycine; Cip = ciprofloxacin; SYN = synergistic interaction; *ADD = additive interaction*; IND = indifferent interaction; ANT = antagonistic interaction.

without the knowledge of their medicinal practitioner. Notably, a further four combinations of plant coumpounds and conventional antibiotics produced additive effects against *P. mirabilis*. All of these combinations contained either chloramphenicol or erythromycin as the antibiotic component, indicating that the plant compounds may inhibit a common bacterial resistance mechanism that renders resistance against these antibiotics (possibly efflux pumps).[40] As these combinations produce greater inhibitory activity than either the individual components, their use may be beneficial in the prevention and treatment of rheumatoid arthritis. No antagonistic interactions were noted against *P. mirabilis*, indicating all of these combinations are safe to use against this *P. mirabilis* strain without decreasing the efficacy of either component.

A variety of interactive effects were evident for the pure plant compound-conventional antibiotic combinations against *K. pneumoniae*. As noted for *P. mirabilis*, the majority (twelve) of the combinations were non-interactive, verifying that they would be safe to use in combination against *K. pneumoniae* without compromising the activity of either component. Additive effects were noted for another four pure plant compound-conventional antibiotic combinations. Notably, the majority of these combinations contained tetracycline as the antibiotic component. As bacterial resistance to tetracycline is most frequently due to bacterial expression of efflux pumps,[39-42] curcumin, lupeol and piperine may have tetracycline efflux pump inhibitory activity. However, this needs to be verified in future studies. Notably, two combinations (both containing erythromycin as the conventional antibiotic component) produced antagonistic results, indicating that combinations containing erythromycin should be avoided for the treatment of *K. pneumoniae* infections.

Three additive interactions were also noted for combinations of the pure plant compounds and the conventional antibiotics against *A. baylyi*. Additionally, four additive interactions were detected against *P. aeruginosa*. With the exception of the additive lupeol-ciprofloxacin combination against *P. aeruginosa*, all other additive interactions against *A. baylyi* and *P. aeruginosa* contained either chloramphenicol or erythromycin as the antibiotic component. As *A. baylyi* and *P. aeruginosa* are triggers of multiple sclerosis in genetically susceptible people, the use of these additive combinations may be beneficial in preventing and treating that disease. All other combinations were non-interactive and are therefore safe to use against these bacteria without compromising the activity of either component of the combination.

Interestingly, one synergistic interaction (lupeol and tetracycline) was detected against *S. pyogenes*. Additionally, four additive combinations were also detected against *S. pyogenes*. The majority of the potentiating combinations (both synergistic and additive) contained the lupeol as the phytochemical component of the combination, with either chloramphenicol, erythromycin or tetracycline as the antibiotic component. Notably, sterol compounds like lupeol insert into cell membranes, thereby altering membrane fluidity and affecting the structures of membrane protein.[43] It is therefore possible that lupeol may increase intracellular antibiotic concentrations in *S. pyogenes*, either by increasing bacterial antibody uptake, or by decreasing bacterial efflux mechanisms. As additive and synergistic combinations provide enhanced antibacterial activity compared to the individual components, the use of these combinations may be useful in the prevention and treatment of rheumatic fever (and other diseases caused by *S. pyogenes* infections). All other combinations were non-interactive. Whilst these combinations may have no additional benefit compared to that of the individual components, they would not reduce each other's effects. No antagonistic interactions were noted against *S. pyogenes*, indicating that all of these combinations are safe to use against this bacterium without decreasing the efficacy of either the phytochemical or antibiotic components.

Toxicity studies

All of the plant compounds and conventional antibiotics were individually screened in the *Artemia* lethality assay (ALA) in 200 µg/mL concentrations. The compounds were only considered toxic if they induced percentage mortalities greater than 50 % (LD_{50}) following 24 hr of exposure to the *Artemia* nauplii.[39] When tested individually, the conventional antibiotics were non-toxic (Table 3). Similarly, none of the pure plant compounds induced mortality significantly different to that of the negative control. When tested together in the ALA, none of the extract-antibiotic combinations produced significantly higher mortality than the negative controls. Furthermore, no individual components or combinations induced >50 % mortality in the ALA. 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 evaluated and quantified the growth inhibitory properties of curcumin, lupeol and piperine against some bacterial triggers of selected autoimmune inflammatory diseases (rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis and rheumatic fever). There are currently no safe and effective cures for any of these diseases. Instead, current treatments aim to alleviate the inflammatory symptoms (heat, pain, swelling) and decrease the patient's discomfort. Whilst these approaches may alleviate patient suffering, they do not block the development of further tissue damage. A more effective therapeutic approach to autoimmune diseases may be to not only directly target the alleviation of patients' discomfort, but to also target the diseases' etiology. Such an approach would not only treat the disease symptoms, but would allow for prevention of further bouts of the autoimmune inflammatory diseases. Interestingly, relatively few studies have focussed on preventing the disease etiology, although several recent studies have begun to examine these early-phase events.[35,36,38] Our study focussed specifically on inhibiting the etiology of selected autoimmune diseases by inhibiting the growth of their bacterial triggers. *Proteus mirabilis* can induce the production of self-reactive antibodies in people genetically susceptible to rheumatoid arthritis.[1] *Klebsiella pneumoniae* can induce ankylosing spondylitis, *A. baylyi* and *P. aeruginosa* can induce multiple sclerosis and *S. pyogenes* can induce rheumatic fever in genetically susceptible people.[1,2] Curcumin, lupeol and piperine each inhibited the growth of all of these bacteria, often with clinically relevant potency (MIC values <1000 µg/mL). The activity of lupeol (266 µg/mL; 625 µM) and piperine (178 µg/mL; 625 µM) against *P. mirabilis* was particularly noteworthy.

Whilst several previous studies have screened curcumin, lupeol and piperine for antibacterial activity, most studies have concentrated on other bacterial species.[27-29,34] Notably, the MIC values determined in several of those studies correlate well with those determined in our study. Indeed, MIC values of 250 µg/mL were previously reported for lupeol *E. coli, S. aureus* and *P. aeruginosa*,[27] compared to an MIC of 356 µg/mL against *P. aeruginosa* in our study. Another study reported that *K. pneumoniae* and *P. aeruginosa* were susceptible to lupeol,[28] but did nor determine MIC values making it difficult to compare with other studies. Similarly, another study determined an MIC of 1250 µg/mL for piperine when tested against *K. pneumoniae*,[34] compared to an MIC of 400 µg/mL in our study. Notably, many of the other studies that screened these compounds for antibacterial activity screened a single, relatively high concentration. The situation for curcumin is less clear as many antibacterial studies have tested turmeric rhizome extracts (containing curcumin) against bacterial pathogens, rather than testing pure curcumin. Some of those studies have reported MIC values as low as 16 µg/mL against multiple bacteria, including *K. pneumoniae*.[17-19]

Table 3: Mortality (%) for curcumin, lupeol, piperine, and conventional antibiotics tested individually and as combinations in the ALA assay.

	Test compound or combination	ALA Mortality ± SEM (%) [a]	
		After 24 hrs:	After 48 hrs:
Negative control	Artificial seawater	3.9 ± 2.1	9.2 ± 3.4
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
	Ciprofloxacin	4.4 ± 2.6	8.3 ± 3.2
Extracts	Curcumin	5.8 ± 3.3	20.6 ± 3.5
	Lupeol	6.7 ± 2.9	25.2 ± 4.4
	Piperine	4.9 ± 1.9	17.6 ± 2.7
Combinations	Curcumin + Penicillin G	6.7 ± 2.2	19.3 ± 3.6
	Curcumin + Chloramphenicol	6.3 ± 3.5	21.2 ± 3.8
	Curcumin + Erythromycin	4.8 ± 2.9	17.5 ± 3.1
	Curcumin + Tetracycline	5.9 ± 2	19.3 ± 3.5
	Curcumin + Gentamicin	7.7 ± 3.2	25.8 ± 3.4
	Curcumin + Ciprofloxacin	8.8 ± 3.7	32.4 ± 4
	Lupeol + Penicillin G	8.3 ± 3.6	24.6 ± 4.2
	Lupeol + Chloramphenicol	7.2 ± 3.4	19.7 ± 3.6
	Lupeol + Erythromycin	6.8 ± 2.6	20.7 ± 2.4
	Lupeol + Tetracycline	7.4 ± 2.3	22.5 ± 2.7
	Lupeol + Gentamicin	10.5 ± 3.4	29.2 ± 3.8
	Lupeol + Ciprofloxacin	13.4 ± 3.7	37.8 ± 3.6
	Piperine + Penicillin G	6 ± 2.5	18.8 ± 3.2
	Piperine + Chloramphenicol	5.4 ± 3	16.6 ± 3.8
	Piperine + Erythromycin	4.7 ± 2.9	13.7 ± 3.1
	Piperine + Tetracycline	6.1 ± 3.2	15.5 ± 3.6
	Piperine + Gentamicin	10.7 ± 3.7	22.5 ± 3.3
	Piperine + Ciprofloxacin	14.9 ± 4.1	31.6 ± 3.7

Results represent means ± SEM of 3 independent experiments, each preformed in triplicate ($n = 9$).

Whilst this may indicate that curcumin has substantial activity against those pathogens, extracts are complex mixtures of compounds that may affect each other's activity substantially.[40] The presence of potentiating compounds in turmeric extracts may increase the potency of curcumin by several fold and substantially more work is required to quantify the effects of the pure compounds and to compare them with the properties of turmeric extracts. Indeed, sesquiterpenoid tumerone compounds in turmeric have been reported to increase curcumin bioavailability by increasing the permeability of curcumin in cell membranes, thereby increasing intracellular concentrations.[44] The same study also reported that tumerones also enhance *in vivo* bioavailability by aiding transport to the target tissue. Therefore combinations of curcumin and tumerones may have substantially better antibacterial activity than curcumin alone, although this remains to be determined.

Whilst our study highlights the potential use of curcumin, lupeol and piperine to prevent some autoimmune diseases by inhibiting the growth of their bacterial triggers, it is noteworthy that the MIC values reported for these compounds is substantially higher than determined for the conventional antibiotic controls. For example, the MIC values determined for curcumin, lupeol and piperine ranged from 178 to 346 µg/mL. In comparison, MIC values 0.3-2.5 µg/mL were determined for the antibiotic controls in our study, which is substantially more potent than the pure plant compounds. MIC values >1 µg/mL for the conventional antibiotics are deemed to indicate resistance to that antibiotic. Thus, whilst these compounds have therapeutic potential, their potency is still relatively low compared to that of the conventional antibiotics. Future studies are required to screen curcumin, lupeol and piperine against extended panels of bacterial strains with different antibiotic susceptibility/resistance profiles.

Additionally, the effects of curcumin, lupeol and piperine may be substantially less *in vivo* than reported in this, and other studies. Curcumin, lupeol and piperine are all relatively nonpolar, and therefore have low bioavailability.[44-47] In addition to their low solubility, only low levels of these compounds are absorbed across the gastrointestinal lumen, and they are rapidly metabolised and eliminated.[47] Therefore, to obtain therapeutic effects *in vivo*, substantially higher concentrations of these compounds may need be ingested orally to obtain clinically useful effects. Much recent work has focussed on methods of increasing bioavailability of these compounds, either by adulteration of the pure compound, or by using crude extracts instead of pure compounds. Alternatively, the use of combinations containing potentiators may allow relatively low levels of these compounds to have enhanced/clinically relevant effects. Substantial further work is needed in these areas.

The combinational studies reported in this report were perhaps more interesting and highlighted several combinations with potentiated antibacterial activity. Indeed, one synergistic and nineteen additive combinations were detected, including potentiation against antibiotic-resistant bacterial strains. The implications of these potentiating combinations include enhanced efficacy and the requirement for lower doses, which may reduce side effects of the treatment.[40] However, most of these combinations produced indifferent interactions and therefore may not have greater benefits than either component separately. However, these findings may alleviate concerns about the use of the two compounds concurrently without reducing the efficacy of the other therapy.

Our study only tested the potential of curcumin, lupeol and piperine to inhibit the etiology of some autoimmune diseases by blocking the bacterial triggers and does not directly test for anti-inflammatory effects. However, all of these compounds have previously been reported to have substantial anti-inflammatory effects via several mechanisms. Each of these compounds inhibit the production and/or release of pro-inflammatory cytokines, whilst upregulating the production of anti-inflammatory cytokines.[11,15,23-26,31,32] Therefore, these compounds may be particularly useful in the prevention and treatment of rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis and rheumatic fever as they block both the trigger events as well as the later phase effects. Further studies are required to determine whether these compounds also have beneficial effects towards other aspects of these diseases.

None of the plant compounds or conventional antibiotics were toxic when tested independently in the ALA toxicity assay. Similarly, all combinations were nontoxic in the ALA assay, indicating their potential for therapeutic use. However, *in vitro* toxicity studies using other human cell lines are required to verify the safety of these compounds prior to

clinical usage. Future studies should also use *in vivo* toxicity assays to confirm the safety of these compounds and combinations in complex biological systems.

CONCLUSION

Whilst the findings reported herein indicate the potential of curcumin, lupeol and piperine to inhibit the etiological events of rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis and rheumatic fever, 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. Additionally, it is unknown whether these compounds can also affect other phases of the progession of these autoimmune diseases and further studies are required to test for these effects.

ACKNOWLEDGEMENT

The financial assistance of the Centre for Planetary Health and Food Security, and the School of Environment and Science, Griffith University is hereby acknowledged.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

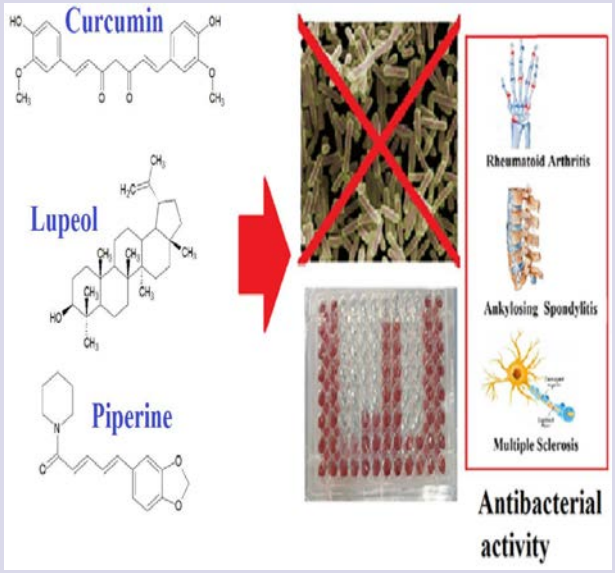
ALA: brine-shrimp lethality assay; **DMSO:** dimethyl sulfoxide; **INT:** ρ-iodonitrotetrazolium chloride; **LD_{50}:** dose of sample necessary to have a lethal effect on 50% of test organisms or cells; **MIC:** minimum inhibitory concentration; **ΣFIC:** the sum of the fractional inhibitory concentration; **ZOI:** zone of inhibition.

REFERENCES

1. 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.
2. 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. Vol. 1: Application of Multiple Sclerosis; 2019. doi: 10.1088/978-0-7503-1762-7ch2.
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. Graham DJ, Campen D, Hui R, Spence M, Cheetham C, Levy G, *et al* Risk of acute myocardial infarction and sudden cardiac death in patients treated with cyclo-oxygenase 2 selective and non-selective non-steroidal anti-inflammatory drugs: Nested case-control study. Lancet. 2005;365(9458):475-81. doi: 10.1016/S0140-6736(05)17864-7, PMID 15705456.
5. Cock IE. The early stages of rheumatoid arthritis: New targets for the development of combinational drug therapies. OA Arthritis. 2014;2(1):5.
6. 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, PMID 16603443.
7. 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, PMID 1466602.
8. 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.
9. 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.
10. Khumalo GP, Van Wyk BE, Feng Y, Cock IE. A review of the traditional use of southern African medicinal plants for the treatment of inflammation and inflammatory pain. J Ethnopharmacol. 2022;283. doi: 10.1016/j.jep.2021.114436, PMID 114436.
11. Hewlings SJ, Kalman DS. Curcumin: A review of its effects on human health. Foods. 2017;6(10):92. doi: 10.3390/foods6100092, PMID 29065496.
12. Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol. 2007;595:105-25. doi: 10.1007/978-0-387-46401-5_3, PMID 17569207.
13. Lin YG, Kunnumakkara AB, Nair A, Merritt WM, Han LY, Armaiz-Pena GN, *et al*. Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. Clin Cancer Res. 2007;13(11):3423-30. doi: 10.1158/1078-0432.CCR-06-3072, PMID 17545551.
14. Marchiani A, Rozzo C, Fadda A, Delogu G, Ruzza P. Curcumin and curcumin-like molecules: From spice to drugs. Curr Med Chem. 2014;21(2):204-22. doi: 10.2174/09298673210213120611 5810, PMID 23590716.
15. Panahi Y, Hosseini MS, Khalili N, Naimi E, Simental-Mendía LE, Majeed M, *et al*. Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial. Biomed Pharmacother. 2016;82:578-82. doi: 10.1016/j.biopha.2016.05.037, PMID 27470399.
16. Zorofchian Moghadamtousi S, Abdul Kadir H, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed Res Int. 2014;2014:Article ID 186864.. doi: 10.1155/2014/186864.
17. Niamsa N, Sittiwet C. Antimicrobial activity of *Curcuma longa* aqueous extract. J Pharmacol Toxicol. 2009;4(4):173-7. doi: 10.3923/jpt.2009.173.177.
18. Ungphaiboon S, Supavita T, Singchangchai P, *et al*. Study on antioxidant and antimicrobial activities of turmeric clear liquid soap for wound treatment of HIV patients. Songklanakarin J Sci Technol. 2005;2005(27 (2)):269-578.
19. Lawhavinit OA, Kongkathip N, Kongkathip B. Antimicrobial activity of curcuminoids from *Curcuma longa* L. on pathogenic bacteria of shrimp and chicken. Kasetsart J Nat Sci. 2010;44(3):364-71.
20. Tajbakhsh S, Mohammadi K, Deilami I, *et al*. Antibacterial activity of indium curcumin and indium diacetylcurcumin. Afr J Biotechnol. 2008;7(21):3832-5.
21. Mun SH, Joung DK, Kim YS, Kang OH, Kim SB, Seo YS, *et al*. Synergistic antibacterial effect of curcumin against methicillin-resistant *Staphylococcus aureus*. Phytomedicine. 2013;20(8-9):714-8. doi: 10.1016/j.phymed.2013.02.006. PMID 23537748.
22. Saleem M. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Lett. 2009;285(2):109-15. doi: 10.1016/j.canlet.2009.04.033, PMID 19464787.
23. Fernández MA, De las Heras B, García MD, Sáenz MT, Villar A. New insights into the mechanism of action of the anti-inflammatory triterpene lupeol. J Pharm Pharmacol. 2001;53(11):1533-9. doi: 10.1211/0022357011777909, PMID 11732756.
24. Fernández A, Alvarez A, García MD, Sáenz MT. Anti-inflammatory effect of *Pimenta racemosa* var. ozua and isolation of the triterpene lupeol. Farmaco. 2001;56(4):335-8. doi: 10.1016/s0014-827x(01)01080-1, PMID 11421264.
25. Bani S, Kaul A, Khan B, Ahmad SF, Suri KA, Gupta BD, *et al*. Suppression of T lymphocyte activity by lupeol isolated from *Crataeva religiosa*. Phytother Res. 2006;20(4):279-87. doi: 10.1002/ptr.1852, PMID 16557610.
26. Harish BG, Krishna V, Santosh Kumar HS, Khadeer Ahamed BM, Sharath R, Kumara Swamy HM. Wound healing activity and docking of glycogen-synthase-kinase-3-β-protein with isolated triterpenoid lupeol in rats. Phytomedicine. 2008;15(9):763-7. doi: 10.1016/j.phymed.2007.11.017, PMID 18222664.
27. Shai LJ, McGaw LJ, Aderogba MA, Mdee LK, Eloff JN Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) CA Sm. leaves. Journal of Ethnopharmacology. 2008;119(2):238-44. doi: 10.1016/j.jep.2008.06.036.
28. Ahamed BKM, Krishna V, Gowdru HB, *et al*. Isolation of bactericidal constituents from the steam bark extract of Grewia tiliaefolia Vahl. Res J Med Plants;20007(1):72-82.
29. Mathabe MC, Hussein AA, Nikolova RV, Basson AE, Meyer JJ, Lall N. Antibacterial activities and cytotoxicity of terpenoids isolated from *Spirostachys africana*. J Ethnopharmacol. 2008;116(1):194-7. doi: 10.1016/j.jep.2007.11.017, PMID 18191928.
30. Haq IU, Imran M, Nadeem M, Tufail T, Gondal TA, Mubarak MS. Piperine: A review of its biological effects. Phytother Res. 2021;35(2):680-700. doi: 10.1002/ptr.6855, PMID 32929825.
31. Zhai WJ, Zhang ZB, Xu NN, Guo YF, Qiu C, Li CY, *et al*. Piperine plays an anti-inflammatory role in *Staphylococcus aureus* endometritis by inhibiting activation of NF-κB and MAPK pathways in mice. Evid Based Complement Alternat Med. 2016;2016:8597208. doi: 10.1155/2016/8597208.
32. Jangra A, Kwatra M, Singh T, Pant R, Kushwah P, Sharma Y, *et al*. Piperine augments the protective effect of curcumin against lipopolysaccharide-induced neurobehavioral and neurochemical deficits in mice. Inflammation. 2016;39(3):1025-38. doi: 10.1007/s10753-016-0332-4, PMID 26970969.
33. Pan H, Xu LH, Huang MY, Zha QB, Zhao GX, Hou XF, *et al*. Piperine metabolically regulates peritoneal resident macrophages to potentiate their functions against bacterial infection. Oncotarget. 2015;6(32):32468-83. doi: 10.18632/oncotarget.5957, PMID 26439699.
34. Zarai Z, Boujelbene E, Ben Salem N, Gargouri Y, Sayari A. Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from *Piper nigrum*. LWT Food Sci Technol. 2013;50(2):634-41. doi: 10.1016/j.lwt.2012.07.036.

35. 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.
36. 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.
37. Tiwana G, Cock IE, White A, Cheesman MJ. Use of specific combinations of the triphala plant component extracts to potentiate the inhibition of gastrointestinal bacterial growth. J Ethnopharmacol. 2020;260:112937. doi: 10.1016/j.jep.2020.112937.
38. Ilanko P, McDonnell PA, Van Vuuren S, Cock IE. Interactive antibacterial profile of *Moringa oleifera* Lam. extracts and conventional antibiotics against bacterial triggers of some autoimmune inflammatory diseases. S Afr J Bot. 2019;124:420-35. doi: 10.1016/j.sajb.2019.04.008.
39. 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.
40. 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.
41. Tintino SR, Morais-Tintino CD, Campina FF, Costa MDS, Menezes IRA, De Matos YMLS, *et al.* Tannic acid affects the phenotype of *Staphylococcus aureus* resistant to tetracycline and erythromycin by inhibition of efflux pumps. Bioorg Chem. 2017;74:197-200. doi: 10.1016/j.bioorg.2017.08.004, PMID 28841421.
42. 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.
43. Sudharsan PT, Mythili Y, Selvakumar E, Varalakshmi P. Lupeol and its ester inhibit alteration of myocardial permeability in cyclophosphamide administered rats. Mol Cell Biochem. 2006;292(1-2):39-44. doi: 10.1007/s11010-006-9171-1, PMID 17009100.
44. Yue GG, Cheng SW, Yu H, Xu ZS, Lee JK, Hon PM, *et al.* The role of turmerones on curcumin transportation and P-glycoprotein activities in intestinal Caco-2 cells. J Med Food. 2012 Mar 1;15(3):242-52. doi: 10.1089/jmf.2011.1845, PMID 22181075.
45. Stanic Z. Curcumin, a Compound from Natural Sources, a True Scientific Challenge - A Review. Plant Foods Hum Nutr. 2017;72(1):1-12. doi: 10.1007/s11130-016-0590-1, PMID 27995378.
46. Cháirez-Ramírez MH, Gallegos-Infante JA, Moreno-Jiménez MR, González-Laredo RF, Rocha-Guzmán NE. Absorption and distribution of lupeol in CD-1 mice evaluated by UPLC–APCI+–MS/MS. Biomed Chromatogr. 2019;33(3):e4432. doi: 10.1002/bmc.4432, PMID 30419143.
47. Zhang W, Zheng Q, Song M, Xiao J, Cao Y, Huang Q, *et al.* A review on the bioavailability, bio-efficacies and novel delivery systems for piperine. Food Funct. 2021;12(19):8867-81. doi: 10.1039/d1fo01971f, PMID 34528635.

PICTORIAL ABSTRACT



SUMMARY

- Curcumin, lupeol and piperine were screened for growth inhibitory activity in the disc diffusion assay against bacterial triggers of some autoimmune diseases.
- The ability to inhibit the growth of some bacterial triggers of selected autoimmune inflammatory diseases was quantified by liquid dilution MIC assays.
- The pure plant compounds were also tested in combination with conventional antibiotics to determine whether the extracts cold potentiate the antibiotic activity.
- Toxicity of the individual compounds and combinations was evaluated using the *Artemia* nauplii bioassay.

About Authors

Dr Ian Cock leads a research team in the Environmental Futures Research Institute and the School of Natural Sciences at Griffith University, Australia. His research involves bioactivity and phytochemical studies into a variety of plant species of both Australian and international origin, including *Aloe vera*, South Asian and South American tropical fruits, as well as Australia plants including *Scaevola spinescens, Pittosporum phylliraeoides*, *Terminalia ferdinandiana* (Kakadu plum), Australian *Acacias, Syzygiums, Petalostigmas* and *Xanthorrhoea johnsonii* (grass trees). This range of projects has resulted in nearly 200 publications in a variety of peer reviewed journals.