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Original Article

Podocarpus latifolius (Thunb.) R.Br. ex Mirb. Extracts Inhibit the Growth Some Bacterial Triggers of Rheumatoid Arthritis and Ankylosing Spondylitis

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ABSTRACT

Introduction: *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb. is a large tree that is native to southern Africa. This species has been used extensively for multiple therapeutic purposes across the southern part of the African continent. Despite this, *P. latifolius* leaf extracts have not been rigorously examined growth inhibitory properties against many bacteria, including the bacterial triggers of autoimmune inflammatory diseases. **Materials and Methods:** The antimicrobial activity of *P. latifolia* leaf solvent extractions was investigated by disc diffusion and growth time course assays against some bacterial triggers of rheumatoid arthritis and ankylosing spondylitis. The growth inhibitory activity was further quantified by MIC determination. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** Methanolic and aqueous *P. latifolia* leaf extracts were good inhibitors of *P. mirabilis* and *K. pneumoniae*. The inhibitory activity of both extracts was particularly noteworthy against the bacterial trigger of ankylosing spondylitis (*K. pneumonia*), with MICs of 343 and 557 µg/mL determined for the methanolic and aqueous extracts respectively. The extracts also displayed noteworthy activity against a bacterial trigger of rheumatoid arthritis (*P. mirabilis*), with MICs of 580 and 634 µg/mL respectively. The antibacterial activity of the methanolic and aqueous *P. latifolia* leaf extracts were further investigated by growth time course assays, which showed significant growth inhibition in cultures of *P. mirabilis* and *K. pneumoniae* within 1 hr of exposure. The extracts were determined to be nontoxic in the *Artemia franciscana* nauplii bioassay, indicating their safety for prophylactic use in preventing these autoimmune inflammatory diseases. **Conclusion:** The lack of toxicity of the *P. latifolia* leaf extracts and their growth inhibitory bioactivity against the bacterial triggers of rheumatoid arthritis and ankylosing spondylitis indicate their potential in the development of new therapies targeting the onset of these diseases.

Keywords: Podocarpaceae, Yellowwood, South African plants, Rheumatoid arthritis, Ankylosing spondylitis, Antibacterial activity, Medicinal plants.

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INTRODUCTION

The autoimmune inflammatory disorders including rheumatoid arthritis and ankylosing spondylitis are debilitating conditions which afflict genetically susceptible individuals. Notably, there are no cures for these conditions. Instead, current treatment strategies aim to alleviate the symptoms (particularly pain, swelling and inflammation) with analgesics and anti-inflammatory agents and/or to modify the disease progression through the use of disease modifying drugs. None of these treatments is ideal as prolonged usage of these drugs is often accompanied by unwanted side effects and toxicity.[1] There is a need to develop safer, more effective treatments for these conditions which will not only alleviate the symptoms, but may also cure or prevent the disease. A greater understanding of the onset and progression of these disorders may assist in more relevant drug discovery and development.

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Although the causes of the autoimmune inflammatory disorders are not well understood, it is generally accepted that they are triggered in susceptible individuals by specific antigens. Recent serotyping studies have identified several bacterial triggers of some of these conditions and the specific antigens responsible for the induction of the immune response.[2-8] The major microbial trigger of rheumatoid arthritis has been identified as *Proteus mirabilis*,[2-4,8] a normal component of the human gastrointestinal microbiome. Similarly, *Klebsiella pneumoniae* can initiate ankylosing spondylitis in genetically susceptible people[2,5-8] and *Acinetobacter baylyi* and *Pseudomonas aeruginosa* have been linked with the onset of multiple sclerosis.[8,9] *Borrelia burgdorferi*

is linked with Lyme disease.[8,10] Whilst microbial triggers have also been postulated for lupus, the specific causative agents are yet to be identified. Members of the Enterobacteriaceae family are associated with Graves' disease and Kawasaki syndrome and *Mycoplasma pneumoniae* is associated with several demyelinating diseases.[8,11] The development of specific antibiotic agents targeted at theses bacterial pathogens may prevent the onset of the disease in afflicted individuals and may reduce the severity of the symptoms once the disease has progressed.

The increased incidence in antibiotic resistant bacterial pathogens (including the bacterial triggers of autoimmune inflammatory diseases) has resulted in the increases in the incidence of extremely (XDR) or Totally drug resistant (TDR) bacteria, which are relatively unaffected by common clinical antibiotics.[12] There is an urgent need to develop new antibiotic chemotherapies as there are now limited therapeutic options for many diseases caused by bacterial pathogens. Indeed, the development of alternative antibacterial treatment modalities has become crucial and is considered by the World Health Organisation (WHO) to be one of the most serious challenges facing medical science.[13] For a number of reasons reviewed elsewhere,[12] it is unlikely that the previous methods of antibiotic discovery/development will be as successful in the future and new treatment modalities are urgently required. A re-examination of traditional medicines for the treatment of bacterial infections is an attractive prospect as the antiseptic qualities of medicinal plants have also been long recognised and recorded. Traditional herbal remedies have great potential for antimicrobial drug development and there has recently been a substantial increase in interest in this field.[14-27]

Podocarpus latifolius (Thunb.) R.Br. ex Mirb. (family Podocarpaceae; commonly known as yellowwood, broad-leaf yellowwood) is a large evergreen tree (Figure 1a) that is native to southern Africa. *Podocarpus latifolius* trees grow to 30 m tall and have strap-shaped leaves that can reach up to 40mm long (Figure 1b). Round seeds develop of thickened fleshy stalks on female trees in July to September and these turn blue-grey and then purple as they mature (Figure 1c). Whilst *P. latifolius* is best known for its quality wood, it also has numerous uses in southern African traditional medicine systems, including for the treatment of fevers, coughs, respiratory diseases, arthritis, sexually transmitted infections etc.[28-33] Notably, several of these diseases are caused by bacterial pathogens and some studies have screened *P. latifolius* extracts against limited panels of bacteria.[30,31] However, those studies are rudimentary and have only tested *P. latifolius* extracts against limited bacterial pathogens and many more bacteria remain to be tested. Furthermore, none of the previous studies have examined the effects of *P. latifolius* extracts on bacterial triggers of autoimmune inflammatory diseases. The current study was undertaken to screen *P. latifolia* leaf extracts for growth inhibitory properties against bacterial triggers of rheumatoid arthritis (*P. mirabilis*) and ankylosing spondylitis (*K. pneumoniae*).

MATERIALS AND METHODS

Plant collection and extraction

Podocarpus latifolius (Thunb.) R.Br. ex Mirb. leaves were obtained from and identified by Philip Cameron, senior botanic officer, Mt Cootha Botanical Gardens, Brisbane, Australia. The leaves were processed in a Sunbeam food dehydrator to ensure that they were thoroughly dehydrated and subsequently ground into a coarse powder. The powdered leaves was extracted by standardised methods.[34,35] Briefly, an amount of 1 g of dried nut powder was weighed into each of five tubes and five different extracts were prepared by individually adding 50mL of AR grade methanol (Ajax Fine Chemicals, Australia) or deionised water. The powdered leaves were individually extracted in each solvent for 24 hr at 4°C with gentle shaking. The extracts were subsequently filtered through Whatman No. 54 filter paper under vacuum, followed by drying by rotary evaporation in an Eppendorf

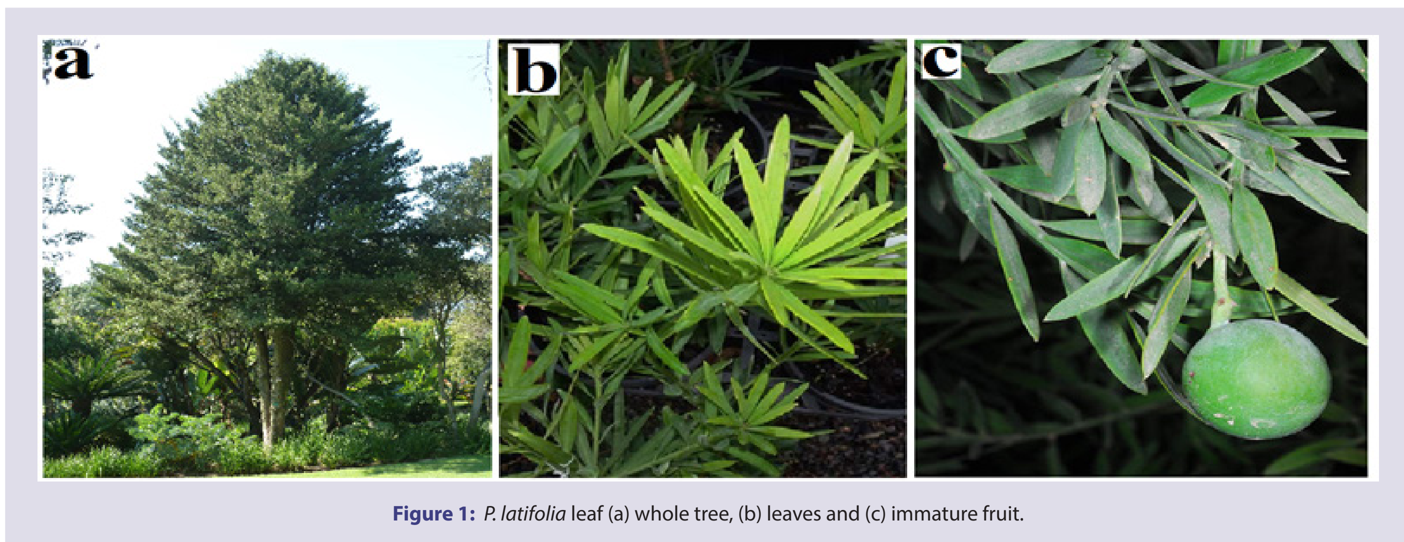

Figure 1: *P. latifolia* leaf (a) whole tree, (b) leaves and (c) immature fruit.

concentrator 5301. The dry extract was weighed to determine the extraction yield and redissolved in 10 mL deionised water (containing 1% DMSO). The extracts were passed through 0.22 µm filter (Sarstedt) and stored at 4ºC until use.

Qualitative phytochemical studies

Phytochemical analysis of the *P. latifolia* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.[36-38]

Figure 1: *P. latifolia* leaf (a) whole tree, (b) leaves and (c) immature fruit.

Antibacterial screening

Test microorganisms

All media was supplied by Oxoid Ltd. Australia. Reference strains of *Klebsiella pneumoniae* (ATCC31488) and *Proteus mirabilis* (ATCC21721) were purchased from American Tissue Culture Collection, USA. Stock cultures were subcultured and maintained in nutrient broth at 4ºC.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified disc diffusion assay.[39-41] Briefly, 100 µL of each bacterial culture was grown in 10 mL of fresh nutrient broth until they reached a count of ~10^8 cells/mL. A volume of 100 µL of the bacterial suspension was spread onto nutrient agar plates and extracts were tested for antibacterial activity using 6 mm sterilised filter paper discs. Discs were infused with 10 µL of the plant extracts, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4ºC for 2 hr before incubation at 37ºC for 24 hr. The diameters of the inhibition zones were measured to the closest whole millimetre. Each assay was performed in triplicate, with three internal replicates (*n*=9). Mean values (±SEM) are reported in this study. Standard discs of ampicillin (10 µg) and chloramphenicol (2 µg) were obtained from Oxoid Ltd., Australia and were 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 (MIC) of each extract was determined as previously described.[40,41] Briefly, the *P. latifolia* leaf extracts were diluted in deionised water and tested across a range of concentrations. Discs were infused with 10 µL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was completed as outlined above and graphs of the zone of inhibition versus Ln of the extract concentration were plotted. Linear regression was used to determine the MIC values of each extract.

Bacterial growth time course assay

Bacterial growth time course studies were performed as previously described.[42] Briefly, 3 mL of *Proteus mirabilis* (ATCC21721) and *Klebsiella pneumoniae* (ATCC31488) in nutrient broth were added individually to 27 mL nutrient broth containing 3 mL of 10 mg/mL methanolic and aqueous plant extract to give a final concentration of 1000 µg/mL in the assay. The tubes were incubated at 30ºC with gentle shaking. The optical density was measured hourly at 550 nm for a 6 hr incubation period. Control tubes were incubated under the same conditions but without the extract. All assays were performed in triplicate.

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 4mg/mL solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay.[43-45] Briefly, 400 µL of seawater containing approximately 48 (mean 47.7, *n*=125, SD 11.4) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 µL of diluted plant extracts or the reference toxin were transferred to the wells and incubated at 25±1ºC under artificial light (1000 Lux). A 400 µL seawater negative control was run in triplicate for each plate. All treatments were performed in at least triplicate, each with three internal repeats (*n*=9). The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was detected within 10 sec. After 24 hr, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95% confidence limits for each treatment was determined using probit analysis.

Statistical analysis

Data are expressed as the mean±SEM of three independent experiments, each with internal triplicates (*n*=9). One way ANOVA was used to calculate statistical significance between control and treated groups with a *p* value <0.01 considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of dried and powdered *P. latifolia* leaf with methanol and deionised water yielded 281 mg and 247 mg of dried extracts respectively (Table 1). The dried extracts were resuspended in 10 mL of deionised water (containing 1%

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

Extract	Mass of Dried Extract (mg)	Resuspended Extract (mg/mL)	Phenols			Cardiac Glycosides	Saponins		Triterpenes	Phytosteroids	Alkaloids			Flavanoids		Tannins		Anthraquinones	
			Total Phenolics	Water Soluble	Water Insoluble	Keller-Kiliani Test	Froth Persistence	Emulsion test	Salkowski Test	Acetic Anhydride Test	Meyers Test	Wagners Test	Draggendoffs Test	Shinoda Test	Kumar test	Ferric Chloride Test	Lead Acetate Test	Free	Combined
Methanol	281	28.1	+++	+++	+	-	+	+	+	-	-	-	-	+++	++	++	++	-	-
Water	247	24.7	+++	+++	-	-	++	+	-	-	-	-	-	+++	+++	++	++	-	-

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

DMSO), resulting in the extract concentrations shown in Table 1. Qualitative phytochemical studies showed that the methanolic and aqueous extracts contained similar classes of molecules, with moderate to high levels of phenolics, flavonoids and tannins detected in both extracts. Lower levels of saponins were also detected. Triterpenes, phytosterols, alkaloids and anthraquinones were absent or below the detection threshold in both extracts.

Antimicrobial activity

To determine the growth inhibitory activity of the *P. latifolia* leaf extracts against the bacterial pathogens, aliquots (10 µL) of each extract were screened in the disc diffusion assay. The methanolic and aqueous *P. latifolia* leaf extracts both inhibited *P. mirabilis* growth (Figure 2), although the Zones of inhibition (ZOIs) were each ≤7.8 mm, which indicates only moderate inhibitory activity. This inhibition was substantially lower than that seen for the ampicillin (10 µg: inhibition zones of 8.8±0.4 mm) and chloramphenicol controls (10 µg: inhibition zones of 12.3±0.3 mm).

The *P. latifolia* leaf extracts also inhibited *K. pneumoniae* growth and the larger ZOIs indicate greater growth inhibition against this bacterium, as judged by ZOIs (Figure 3). Indeed, the inhibitory activity of the extracts was comparable to that of the ampicillin (10.3±0.3 mm) and chloramphenicol controls (8.8±0.4 mm). The extracts are crude mixtures of compounds and it is likely that the antibacterial components account for only a small % of the total mass of extracted compounds, whereas ampicillin and chloramphenicol are pure compounds. Therefore, the activity of the *P. latifolia* leaf extracts is particularly noteworthy and it is likely that purifying the bioactive components may result is substantially enhanced inhibitory activity. As *K. pneumoniae* has been shown to initiate ankylosing spondylitis,[2,5-8] our results indicate the potential of the *P. latifolia* leaf extracts in the prevention and treatment of that disease and further studies are required to isolate and identify the active component(s).

The antimicrobial efficacy was further quantified by determining the MIC values for each extract against both bacterial pathogens. Noteworthy MIC values were obtained for both the methanolic and aqueous *P. latifolia* leaf extracts against both bacterial triggers of autoimmune diseases (as judged by MIC; Table 2). *Klebsiella pneumoniae* was particularly susceptible to the *P. latifolia* leaf extracts, with MIC values of 343 and 557 µg/mL for the methanolic and aqueous extracts respectively (~3.4 and 5.6 µg of extract infused into the disc respectively). The extracts were similarly good inhibitors of *P. mirabilis*, with MIC values of 580 and 634 µg/mL respectively (~5.8 and 6.3 µg infused into the discs). The noteworthy inhibition of *P. mirabilis* and *K. pneumoniae* further supports the potential of the *P. latifolia* leaf extracts for the prevention of rheumatoid arthritis and ankylosing spondylitis in genetically susceptible individuals.

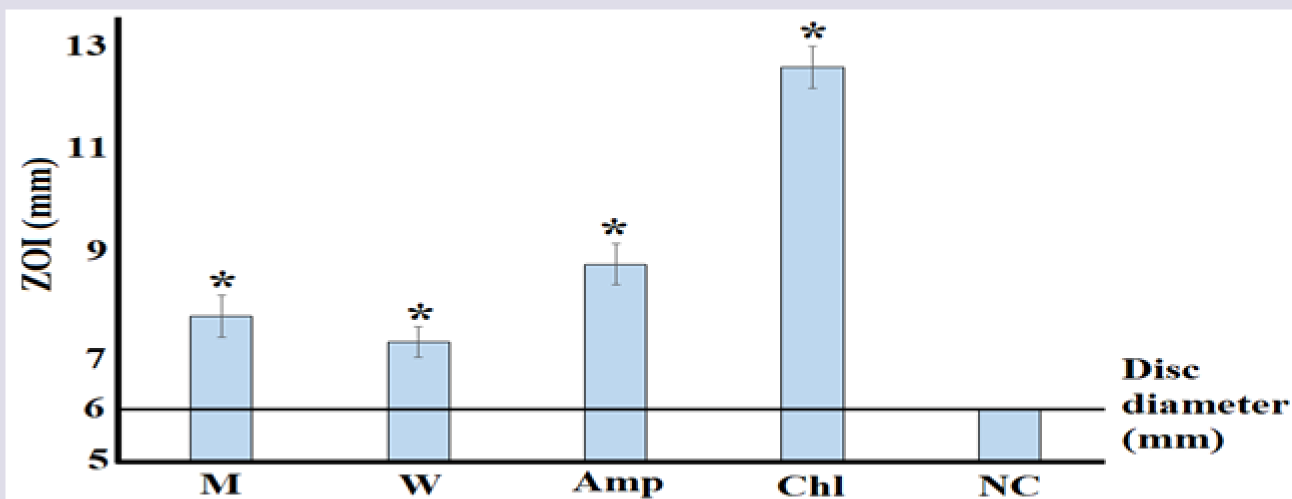

Figure 2: Growth inhibitory activity of *P. latifolia* leaf extracts against *P. mirabilis* (ATCC21721) measured as zones of inhibition (mm)±SEM. M=methanolic extract; W=Aqueous extract; Amp=Ampicillin (10 µg) control; Chl=Chloramphenicol control (2 µg); NC=Negative control. All determinations were performed in triplicate, each with internal triplicates (n=9) and the results are expressed as mean zones of inhibition (mm)±SEM. * indicates results that were significantly different to the negative control (p<0.01).

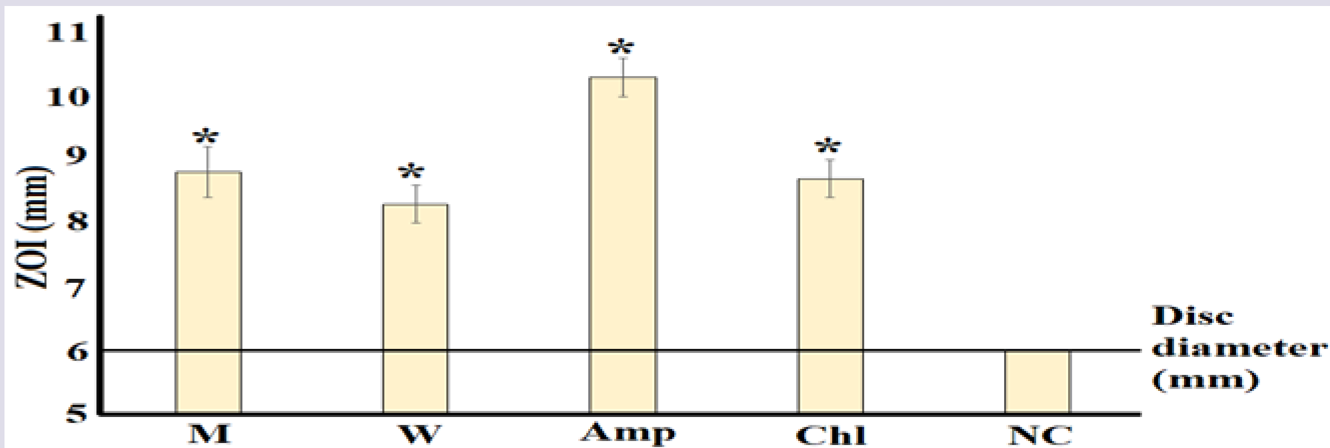

Figure 3: Growth inhibitory activity of *P. latifolia* leaf extracts against *K. pneumoniae* (ATCC31488) measured as zones of inhibition (mm)±SEM. M=methanolic extract; W=Aqueous extract; Amp=Ampicillin (10 µg) control; Chl=Chloramphenicol control (2 µg); NC=Negative control. All determinations were performed in triplicate, each with internal triplicates (n=9) and the results are expressed as mean zones of inhibition (mm)±SEM. * indicates results that were significantly different to the negative control (p<0.01).

Table 2: Minimum inhibitory concentrations (µg/mL) of the *P. latifolia* leaf extracts against each bacterial species and LC_{50} values (µg/mL) against *Artemia* nauplii.

	Bacteria	Strain or exposure time	M	W
Antibacterial Inhibition (MIC)	*P. mirabilis*	ATCC21721	580	634
	K. pneumoniae	ATCC31488	343	557
Toxicity	LC_{50} (µg/mL)	*Aretmia franciscana* nauplii	CND	CND

Numbers indicate the mean MIC or LC_{50} values of at least triplicate determinations. - indicates no growth inhibition or toxicity. M=Methanolic extract; W=Aqueous extract. CND indicates that an LC_{50} could not be determined as the mortality did not exceed 50% at any concentration tested.

Bacterial growth time course assay

The antibacterial activity of the *P. latifolia* leaf extracts was further investigated in *P. mirabilis* (Figure 4a) and *K. pneumoniae* (Figure 4b) by bacterial growth time course assays in the presence and absence of the extract. The starting concentration of the extract used in these assays was 1000 µg/mL. Both the methanolic and aqueous *P. latifolia* leaf extracts significantly inhibited *P. mirabilis* (Figure 4a) and *K. pneumoniae* (Figure 4b) growth within 1 hr, indicating a rapid antimicrobial action. Furthermore, both *P. latifolia* leaf extracts continued to inhibit the growth of *P. mirabilis* and *K. pneumoniae* for the entire period of the time course study, with growth still significantly inhibited following 6 hr. This may indicate that these extracts have bactericidal activity against these bacteria at the dose tested, rather than bacteriostatic activity. Indeed, the turbidity at 6h was not greatly increased from the starting turbidity for either bacteria.

Quantification of toxicity

The toxicity of the *P. latifolia* leaf extracts was initially tested in the *Artemia franciscana* nauplii bioassay at a concentration of 2000 µg/mL (Figure 5). Both the methanolic and aqueous extracts induced low levels of mortality at 24 hr, not substantially greater than the % mortality seen for the seawater control. As neither extract induced >50% toxicity following 24 hr exposure, it was not possible to determine LC_{50} values (Table 2) and both extracts

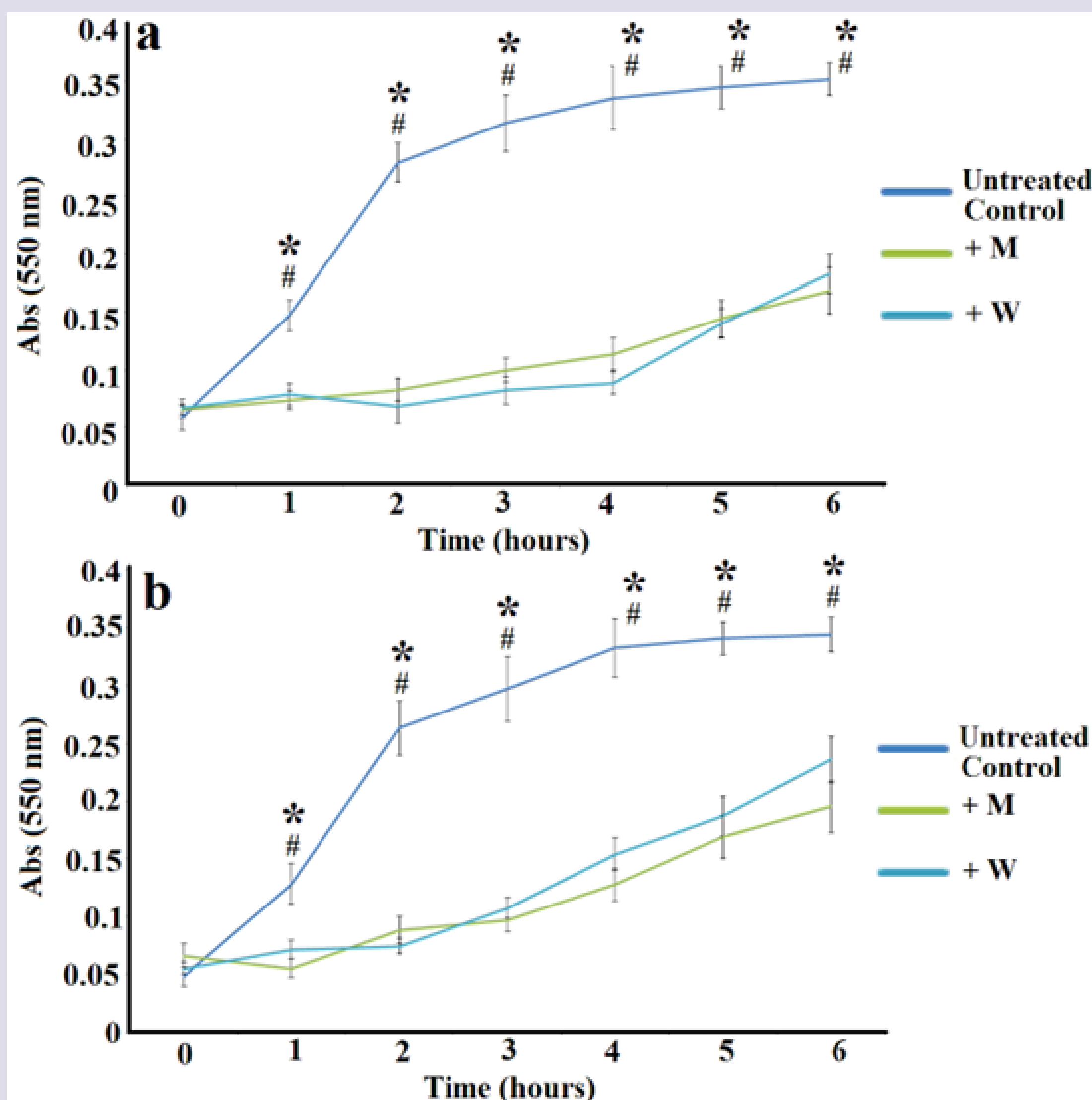

Figure 4: Bacterial growth curves for the methanolic and aqueous *P. latifolia* extracts against (a) *P. mirabilis* (ATCC21721) and (b) *K. pneumoniae* (ATCC31488). All bioassays were performed in triplicate, each with internal triplicates (*n*=9) and the results are expressed as mean±SEM. *=results in the presence of the methanolic extract that are significantly different between the treated and the untreated control growth (*p*<0.01); #=results in the presence of the aqueous extract that are significantly different between the treated and the untreated control growth (*p*<0.01).

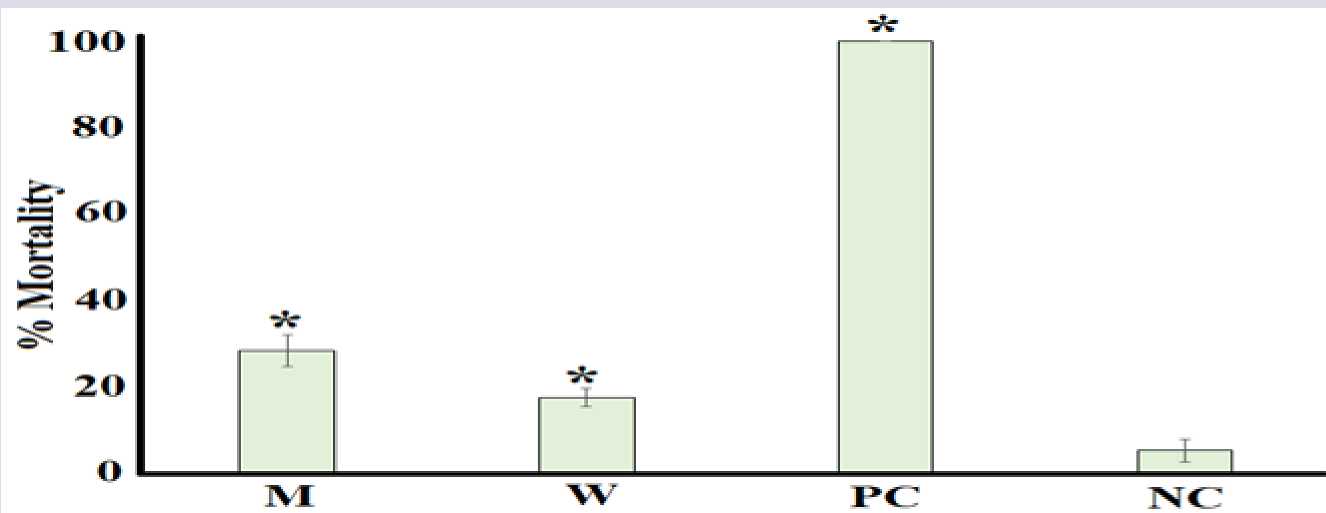

Figure 5: The lethality (expressed as % mortality) of the *P. latifolia* leaf extracts (2000 µg/mL), potassium dichromate (1000 µg/mL) and a seawater control. M=Methanolic extract; W=Aqueous extract; PC=Positive control (1000 µg/mL potassium dichromate); NC=Negative (seawater) control. All bioassays were performed in triplicate, each with internal triplicates (n=9) and the results are expressed as mean±SEM. * indicates results that were significantly different to the negative control ($p<0.01$).

were deemed to be nontoxic.[44,45] In contrast, the potassium dichromate positive control induced mortality within 4 hr (results not shown), with 100% mortality induction seen by 24 hr.

DISCUSSION

Plant remedies are becoming increasingly sought after in the treatment of a myriad of diseases and disorders due both to their perception of greater safety than many synthetic drugs and the failure of current drug regimens to effectively treat many diseases. This is especially true for chronic disorders such as the autoimmune inflammatory diseases. The current treatments utilising Disease modifying anti-rheumatic drugs (DMARDs) to alleviate the symptoms of these diseases and/or alter the disease progression are not entirely effective and have been associated with numerous adverse effects.[46] Furthermore, many of the current treatments are aimed at treating the symptoms without addressing the underlying causes and pathogenic mechanisms. Thus, tissue damage associated with these diseases may still occur. A better understanding of the mechanisms for initiation and progression of the autoimmune inflammatory diseases is important for developing new drugs to target specific processes and thus to more effectively treat autoimmune inflammatory diseases. The studies reported herein examined the ability of *P. latifolia* leaf extracts to block microbial triggers of two autoimmune inflammatory disorders (*P. mirabilis*: rheumatoid arthritis; *K. pneumonia*: ankylosing spondylitis). Both the methanolic and aqueous extracts were identified as potent inhibitors of these bacteria, indicating that they may be useful in the prevention of rheumatoid arthritis and ankylosing spondylitis and in their treatment once they are triggered.

Whilst a detailed investigation of the phytochemistry of the *P. latifolia* leaf extracts was beyond the scope of our study, qualitative screening studies were used to determine the classes of compounds present. Our qualitative phytochemical screening studies indicate that polyphenolics, flavonoids, saponins and tannins were present in relative abundance in the *P. latifolia* leaf extracts. Studies have linked polyphenolic compounds in different plant species with anti-bacterial activity.[47] Thus these compounds may be responsible (at least in part) for the bacterial growth inhibitory activities reported herein. Interestingly, several of these phytochemical classes have also been reported to suppress NF-κB signaling.[47] Thus, these extracts may have pleuripotent effects against autoimmune inflammatory diseases, with therapeutic properties against the trigger events as well as the later inflammatory responses. Further phytochemical evaluation studies and bioactivity driven isolation of the active components is required to evaluate the mechanism of bacterial growth inhibition.

Although these studies have demonstrated the potential of the *P. latifolia* leaf extracts to prevent and treat autoimmune disease, much more work is required. This study has only tested these extracts against microbial triggers of two autoimmune diseases (rheumatoid arthritis and ankylosing spondylitis). The microbial triggers for several other autoimmune inflammatory disorders are also known. *Streptococcus pyogenes* can induce rheumatic heart disease in genetically susceptible people,[8] *Acinetobacter baylyi* and *Pseudomonas aeruginosa* have been linked with the onset of multiple sclerosis.[8] *Borrelia burgdorferi* is linked with Lyme disease[8] and *Mycoplasma pneumoniae* is associated with several demyelinating diseases.[8] It would be interesting to extend

our studies to also screen for the ability of the extracts to block these microbial triggers of autoimmune diseases.

The findings reported herein also demonstrate that the *P. latifolia* leaf extracts were nontoxic towards *Artemia franciscana* nauplii, with LC_{50} values substantially >1000 μg/mL. Extracts with LC_{50} values>1000 μg/mL towards *Artemia* nauplii have previously been defined as nontoxic.[44,45] Whilst our preliminary toxicity studies indicate that these extracts may be safe for therapeutic use, studies using human cell lines are required to further evaluate the safety of these extracts. Furthermore, whilst these studies have demonstrated the potential of the *P. latifolia* leaf extracts in the development of future antibiotic chemotherapeutics for the prevention and treatment of autoimmune diseases (particularly rheumatoid arthritis and ankylosing spondylitis), more work is required to isolate the inhibitory components and determine the mechanism of inhibition.

CONCLUSION

The results of this study demonstrate the potential of the *P. latifolia* leaf as inhibitors of the growth of bacterial species associated with the onset of rheumatoid arthritis and ankylosing spondylitis. Furthermore, their lack of toxicity indicates than they are safe for internal as well as topical treatment.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DMSO: Dimethyl sulfoxide; **LC_{50}:** The concentration required to achieve 50% mortality; **MIC:** Minimum inhibitory concentration.

SUMMARY

• *P. latifolia* leaf extracts inhibited the bacterial triggers of rheumatoid arthritis (*Proteus* spp.) and ankylosing spondylitis (*K. pneumoniae*).

• The methanolic and aqueous extracts were good inhibitors of *P. mirabilis* spp. growth (MICs 580 and 634 μg/mL respectively).

• The methanolic and aqueous extracts were good inhibitors of *K. pneumoniae* growth (MICs 343 and 557 μg/mL respectively).

• The aqueous extracts also had noteworthy bacterial growth inhibitory activity, albeit with slightly higher MIC values.

• All *P. latifolius* extracts were nontoxic in the *Artemia* lethality assay.

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