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Original Article

Jacksonia scoparia R. Br. Leaf Extracts Lack Antibacterial Activity and are Non-toxic *in vitro*

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ABSTRACT

Introduction: Due to the development of bacterial strains that are resistant to multiple antibiotics, the development of new antibiotic therapies has become a priority for medical research. Traditional plant medicines are important leads for the discovery of new therapies and the search for novel antibiotic plant-based treatments has received substantial recent attention. The family Fabaceae is widely used therapeutically in many areas of the world, including for the treatment of bacterial diseases. Despite this, many members of this family are yet to be examined extensively for therapeutic properties. The endemic Australian species *Jacksonia scoparia* R.Br. was screened for antibacterial activity in this study against a panel of bacterial pathogens. **Materials and Methods:** The growth inhibitory activity of *J. scoparia* leaf extracts against a panel of bacterial pathogens was investigated by disc diffusion assay. Toxicity was examined using the *Artemia franciscana* lethality assay (ALA). **Results:** The methanolic and aqueous *J. scoparia* leaf extracts were devoid of inhibitory activity against panels of gram-positive and gram-negative bacteria. The extracts were non-toxic following 24 hr exposure in the ALA assay. **Conclusion:** The *J. scoparia* leaf extracts lacked bacterial growth inhibitory activity. However, these extracts may have other therapeutic properties and testing against protozoa, fungi, virus and tumour cells is required.

Keywords: Fabaceae, Dogwood, Antibiotic resistance, Australian plant, Traditional medicine, Antibacterial activity, Medicinal plants, Toxicity.

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INTRODUCTION

The recent increases in the incidence of antibiotic resistant bacteria has made the development of new antibiotic therapies urgent. Bacteria that are extremely (XDR) or totally drug resistant (TDR) to common clinical antibiotics are now relatively common. Indeed, several bacterial strains have now been reported to be resistant to all current antibiotics.[1] There are now limited therapeutic options for diseases caused by some bacterial pathogens and this is likely to worsen as bacteria exchange resistance genes and more XDR species develop. The discovery of new antibacterial treatment modalities is now crucial and is considered by the World Health Organisation (WHO) to be one of the most serious challenges facing medical science.[2] For a number of reasons reviewed elsewhere,[1] 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. Traditional herbal therapies have great potential for the development of novel antibiotic remedies and there has been a substantial increase in interest in this field.[3-7]

Jacksonia scoparia R.Br. (synonyms *Piptomeris scoparia* (R.Br. ex Sm.) E. Greene, *Jacksonia macrocarpa* Benth., *Jacksonia lateriflora* (Link) R.Br. ex Steudel; commonly known as dogwood) is a small Australian shrub or tree that grows up to 12 metres high (Figure 1a). It is native to the South East Queensland region of Australia and eastern New South Wales. It has small leaves that are most prevalent on young plants or on the base of branches on older specimens (Figure b). *Jacksonia scoparia* produces small bright yellow pea-like flowers (Figure b). Little evidence is available about the traditional use of *J. scoparia* medicinally and we were unable to find any studies examining the antibacterial activity of this species against any bacterial pathogens. Similarly, there is a lack of information on the phytochemical composition of this species. This study was undertaken to screen *J. scoparia* leaf extracts against panels of gram-positive and gram-negative bacterial pathogens.

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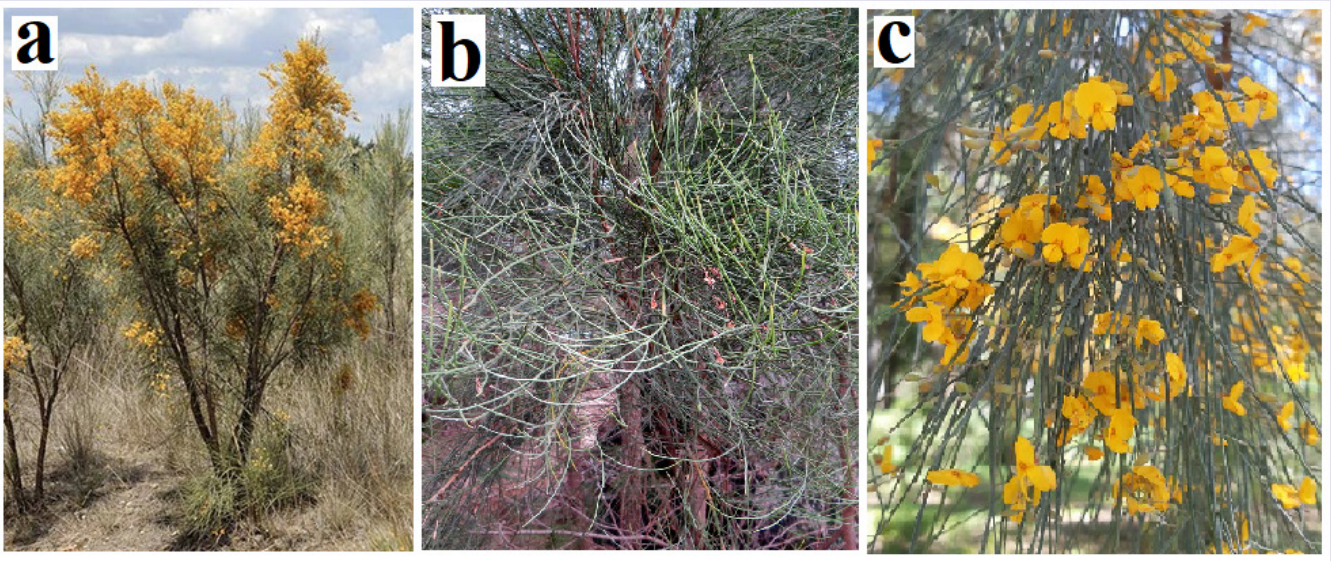

Figure 1: *J. scoparia* (a) whole plant, (b) leaves and (c) flowers. Photographs were taken in 2014 in Toohey Forest by Dr Ian Cock.

MATERIALS AND METHODS

Plant Material

Collection of Plant Material and Extraction

Jacksonia scoparia R.Br. leaves were harvested from a confirmed plant in Toohey Forest, Australia. The harvested leaves were washed in deionised water and processed within 4 hr of collection. The leaves were dried in a Sunbeam food dehydrator and the dried material was ground to a coarse powder. Individual 1g masses of the dried plant material was extracted extensively in 50mL methanol (Ajax Fine Chemicals, AR grade) or sterile deionised water for 24 hr at 4°C with gentle shaking. The extracts were filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation. The resultant pellet was dissolved in 5mL deionised water. The extract was passed through 0.22µm filter (Sarstedt) and stored at 4°C.

Qualitative Phytochemical Studies

Phytochemical analysis of the *J. scoparia* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by standard assays.[8-10]

Antibacterial Screening

Test Microorganisms

All media was purchased from Oxoid Ltd., Australia. The reference strains of *E. coli* (ATCC157293), *Klebsiella pneumoniae* (ATCC31488), *Proteus mirabilis* (ATCC21721) and *Streptococcus pyogenes* (ATCC19615) were purchased from American Tissue Culture Collection (ATCC), USA. Clinical isolate microbial strains of *Aeromonas hydrophilia, Alcaligenes feacalis, Bacillus cereus, Citrobacter freundii, Pseudomonas fluorescens, Salmonella newport, Serratia marcescens, Shigella sonneii, Staphylococcus aureus* and *Staphylococcus epidermidis* were obtained from Ms Michelle Mendell and Ms Jane Gifkins, Griffith University. All stock cultures were subcultured and maintained in nutrient broth at 4°C.

Evaluation of Antimicrobial Activity

Antimicrobial activity of the *J. scoparia* leaf extracts was determined using a modified disc diffusion assay.[11-13] Briefly, 100µL of the each bacterial suspension in log phase was spread onto individual nutrient agar plates and the extracts were tested for antibacterial activity using 6mm sterilised filter paper discs. The discs were each infused with 10µL of the individual plant extract, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4°C for 2 hr before incubation at 37°C for 24 hr. The diameters of the zones of inhibition (ZOIs) were measured to the closest whole millimetre. Each assay was performed three times in triplicate (n=9). Mean values (± SEM) are reported in this study. Standard discs of ampicillin (10µg) and chloramphenicol (10µg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial activity. Filter discs infused with 10µL of distilled water were used as a negative control.

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

			Methanol extract	Water extract
	Mass of extracted material (mg)		295	264
	Concentration of resuspended extract (mg/mL)		29.5	26.4
Qualitative Phytochemical Tests	Phenols	Total phenols	+++	+++
		Water soluble phenols	+++	+++
		Insoluble phenols	++	++
	Saponins	Froth persistence	++	++
		Emulsion test	++	++
	Cardiac glycosides	Keller-Kiliani Test	-	-
	Triterpenoids	Salkowski Test	+	-
	Phytosterols	Acetic Anhydride Test	-	-
	Alkaloids	Meyer's Test	-	-
		Wagner's Test	-	-
		Draggendoff's Test	-	-
	Flavonoids	Kumar Test	+++	+++
	Tannins	Ferric Chloride Test	+	+
		Lead Acetate Test	+	+
	Anthraquinones	Free	-	-
		Combined	-	-

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

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay.[14,15] Briefly, *A. franciscana* nauplii were incubated in the presence of the *J. scoparia* extracts, the reference toxin (1mg/mL potassium dichromate) or artificial seawater (negative control) at 25±1°C under artificial light. All treatments were performed three times in triplicate (n=9). The number of dead were counted in each well at 24 hr, 48 hr and 72 hr. At the completion of the 72 hr exposure period, the remaining live nauplii were sacrificed and the total number of nauplii in each well were counted and used to calculate the % mortality per well. LC_{50} values were calculated for each treatment using probit analysis.

Statistical analysis

Data are expressed as the mean ± SEM of three independent experiments 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 1g of dried and powdered *J. scoparia* leaves with the solvents yielded 295 and 264mg of extracted material respectively (Table 1). The extracts were resuspended in 10mL of deionised water (containing 1% DMSO), resulting in an extract concentrations shown in Table 1. Qualitative phytochemical studies showed that both extracts had similar phytochemical profiles. Both contained high levels of phenolic compounds and flavonoids, as well as moderate levels of saponins and lower levels of triterpenoids and tannins. Cardiac glycosides, phytosterols, alkaloids and anthraquinones were completely absent or below the detection thresholds for these assays.

Antibacterial activity

To determine the growth inhibitory activity of the *J. scoparia* leaf extracts, aliquots (10µL) of each extract were screened in the disc diffusion assay. The *J. scoparia* leaf extracts were ineffective at inhibiting the growth of all gram-negative (Figure 2) and gram positive (Figure 3) bacterial species tested. In contrast, both of the positive control antibiotics (ampicillin and chloramphenicol) were effective growth inhibitors, with ZOI's of up to 14.3mm (ampicillin against *A. faecalis*). We were therefore unable to determine the MIC values for any of the *J. scoparia* extracts as they were completely ineffective at all concentrations tested.

Quantification of Toxicity

The toxicity of the *J. scoparia* leaf extracts was initially tested at 2mg/mL in the *A. franciscana* nauplii bioassay (Figure 4). The mortality in the presence of both extracts was not significantly

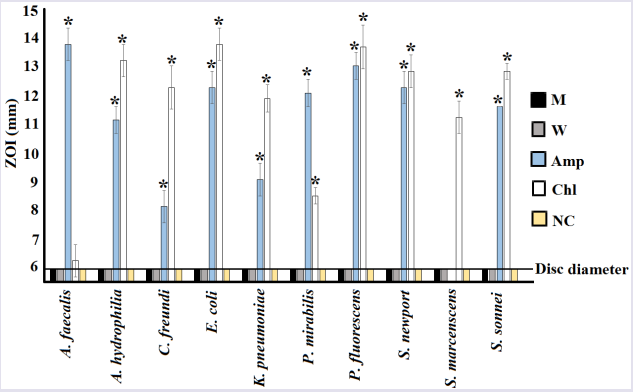

Figure 2: Growth inhibitory activity of *J. scoparia* leaf methanolic and aqueous extracts and reference antibiotics against gram-negative bacterial species measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. M = methanolic extract; W = aqueous extract; NC = negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM.

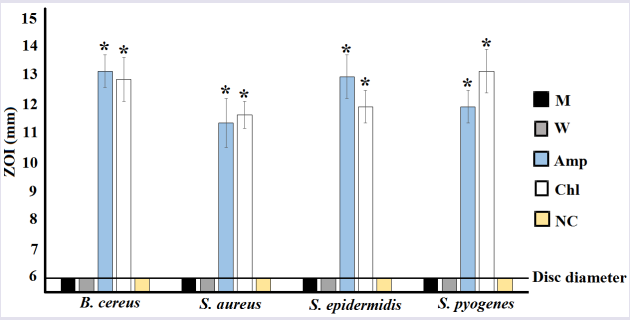

Figure 3: Growth inhibitory activity of *J. scoparia* methanolic and aqueous leaf extracts and reference antibiotics against gram-positive bacterial species measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. M = methanolic extract; W = aqueous extract; NC = negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM.

different to that of the untreated control at 24 hr and thus both extracts were deemed to be non-toxic. Extracts with 24 hr LC_{50} values >1000µg/mL have previously been defined as non-toxic.[14] In contrast, the potassium dichromate positive control induced substantial mortality within 4 hr (results not shown), with 100% mortality induction seen by 24 hr. The mortality increased following exposure to the *J. scoparia* leaf extracts at 48hr and was further increased following 72 hr exposure.

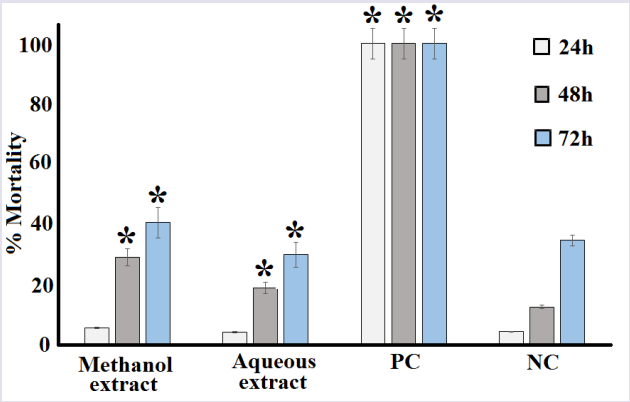

Figure 4: The lethality of the *J. scoparia* methanolic and aqueous leaf extracts, potassium dichromate positive control (1000µg/mL) and seawater (negative control) following 24, 48 and 72 hr of exposaure. PC = positive control; NC = negative control. All bioassays were performed three times in triplicate (*n*=9) and are expressed as mean ± SEM. * indicates results that are significantly different to the untreated (seawater) control at the equivalent exposure time ($p<0.01$).

DISCUSSION

With increasing bacterial resistance towards clinical antibiotics, the development of new antibiotic chemotherapies to inhibit the growth of bacterial pathogens has become a high priority for drug discovery.[1,2] A concurrent decrease in the discovery of new antibiotic medicines by conventional strategies has resulted in an increased interest in traditional medicine plants for the development of novel antibiotic chemotherapies.[16] Whilst we were unable to find records of medicinal usage of *J. scoparia* by the first Australians, it is taxonomically related to other Fabaceae species with antibacterial activity[17] and it was therefore deemed a viable target for antibacterial screening. However, the *J. scoparia* methanolic and aqueous leaf extracts were completely ineffective growth inhibitors against all gram-positive and gram-negative bacterial pathogens tested.

It is noteworthy that a single assay technique was used to screen for antibacterial activity in this study. We chose to use the disc diffusion assay as it is a rapid methodology and it has previously been widely utilised in other studies. Therefore, comparisons between studies are relatively simple. However, as the disc diffusion method is reliant on the diffusion of a molecule through the aqueous environment of an agar gel, this assay may be affected by the solubility of the extract compounds in the aqueous environment. Polar compounds that are highly soluble in water would be expected to diffuse easily in the gel, whereas less soluble compounds would not diffuse as readily and thus be concentrated around the disc. For this reason, whilst this is a handy assay for screening aqueous extracts, this technique may not be ideal for nonpolar compounds (e.g. when screening essential oil and their components). For examining nonpolar mixtures, other techniques such as liquid dilution assays may be preferred. Thus, liquid dilution studies may be better suited to screen *J. scoparia* leaf extracts for activity and future studies will

use these techniques to re-examine the extracts for antibacterial activity.

Diffusion of molecules within an agar gel is also affected by the size of the molecules. The movement of large, complex phytochemicals (eg. complex tannins) through agar gels by diffusion would also be retarded and may provide a false idea of the efficacy of an extract. As many saponins have well described antibiotic properties, screening for growth inhibition using agar diffusion techniques may give a distorted view of its inhibitory potential.

The findings reported here also indicate that the extracts examined were non-toxic (24 hr LC_{50} >1000 µg/mL) in the *Artemia* nauplii bioassay. Whilst toxicity was assessed in this study with the test organism *A. franciscana*, toxicity towards *A. franciscana* has previously been shown to correlate well with toxicity towards human cells for many toxins.[14] However, further studies are required to determine whether this is also true for the *J. scoparia* extracts examined in this study.

CONCLUSION

Methanolic and aqueous *J. scoparia* leaf extracts displayed no antibacterial activity in the disc diffusion assay against a panel of human pathogenic bacteria and were non-toxic towards *Artemia* nauplii.

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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; **ZOI:** Zone of inhibition.

SUMMARY

- *J. scoparia* leaf extracts was screened for the ability to block the growth of a panel of human bacterial pathogens.
- No inhibitory activity was evident against any of the bacterial species tested.
- Toxicity of the *J. scoparia* leaf extracts was determined using the *Artemia* nauplii toxicity bioassay.
- Both the leaf and fruit extracts were non-toxic.

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