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Original Article

The Growth Inhibitory Activity of *Tasmannia lanceolata* (Poir.) A.C. Sm against the Food-poisoning Pathogen *Yersinia enterocolitica*

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ABSTRACT

Introduction: *Yersinia enterocolitica* is a major source of food poisoning via the consumption of contaminated meat products, causing acute gastroenteric yersiniosis. *Tasmannia lanceolata* has been widely documented for its antiseptic properties, repressing the growth of an extensive range of bacteria. Despite this, *Tasmannia lanceolata* has yet to be been tested for its inhibitory capacity against *Y. enterocolitica*. **Methods:** *T. lanceolata* leaf and berry extracts were prepared by maceration and growth inhibitory activity against a clinical strain of *Y. enterocolitica* was examined by disc diffusion assays. The MIC values of the extracts were determined to quantify and compare their relative efficacies. Toxicity was determined using an *Artemia franciscana* nauplii bioassay. **Results:** *T. lanceolata* leaf and berry extracts displayed potent growth inhibitory activity in the disc diffusion assay against *Y. enterocolitica*. The ethyl acetate and chloroform leaf extracts (MICs of 30 and 53 µg/mL respectively) and the hexane berry extract (MIC = 34 µg/mL) were particularly potent growth inhibitors. The methanol and water extracts of both the berry and leaf, as well as the leaf ethyl acetate extract, also had strong growth inhibitory activity against *Y. enterocolitica*, albeit with a higher MIC values (250-300µg/mL). All other extracts had lower efficacy, although their MIC values also indicated good inhibitory activity (with the exception of the chloroform berry extract). When assessed for toxicity, all *T. lanceolata* extracts were non-toxic (LC_{50} values >1000 µg/mL) in the *Artemia franciscana* bioassay. **Conclusion:** The non-toxicity of the *T. lanceolata* berry and leaf extracts, combined with the potent inhibitory bioactivity observed against *Y. enterocolitica*, demonstrates their potential as therapeutic agents in the prevention and treatment of yersiniosis.

Key words: *Yersinia enterocolitica*, Yersiniosis, Antioxidant, Zoonotic, *Tasmannia lanceolata*, Tasmanian pepper.

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INTRODUCTION

Tasmannia lanceolata (Poir.) A.C.Sm (Tasmanian pepper) is a shrub indigenous to the woodlands and cool temperate rainforests of Tasmania and the south-eastern coast of the mainland Australia, to the north of Sydney.[1] It is a medium-large shrub of approximately 2-5 m in height. The berries leaves and bark of this plant are traditionally used medicinally. Australian Aborigines used *T. lanceolata* as a therapeutic agent to treat stomach disorders.[1,2] *T. lanceolata* has also been used traditionally for the treatment and cure of colic, venereal diseases, skin disorders and stomach aches.[2] European colonists also documented the therapeutic properties of *T. lanceolata* and the bark was commonly used as a substitute for other herbal remedies and to treat scurvy (due to its high anti-antioxidant content.[2,3] Despite its extensive ethnobotanical usage, limited scientific studies into the therapeutic properties of *T. lanceolata* have been performed. It has been theorised that the plant's high antioxidant capacity may provide therapeutic benefits. Indeed, investigations of *T. lanceolata* within our laboratory have highlighted the inhibition of bacterial growth by *T. lanceolata* berries, leaves and peppercorns against panels of several distinct pathogenic and food spoilage bacteria[4-6] and protozoal parasites.[7]

The genus *Yersinia* (Y.) includes over than a dozen species of facultative anaerobes.[8] Members of the genus are widespread in the environment but are also extensively present within animal populations. These bacteria are clinically important opportunistic pathogens and can cause human illness. The zoonotic aspect of these bacteria also poses a unique set of challenges in preventing infection, as they can persist in non-human hosts for an indefinite period until they encounter new hosts to infect. Diseases caused by *Yersinia* spp. vary in extremity and mortality, from the bubonic/pneumonic plagues (*Yersinia pestis*) to the yersiniosis (*Yersinia enterocolitica*).[9,10]

Yersinosis is an acute, bacterial gastroenteric infection and accounts for >100,000 illnesses annually in the United States.[11] Characterized by abdominal aching, fever and/or diarrhoea, *Y. enterocolitica* infections frequently originate through the ingestion of infected food/ water. Indeed, almost 90% of all reported cases originate from this mode of infection.[12] The prevention of yersiniosis is complicated by the fact that *Y. enterocolitica* can grow at 4ºC. Therefore, refrigeration alone does not afford adequate protection from contracting the illness.[13] Although it is seldom life-threatening, the economic drain caused through temporary incapacitation from yersiniosis makes probing for effective treatment strategies particularly important. One tactic involves the utilization of natural resources such as plants that have been documented for their antibacterial capabilities. These relatively untapped reservoirs may impede *Y. enterocolitica* growth and thus may offer new management options for yersiniosis. Despite the documented ability of *T. lanceolata* to inhibit the growth of many bacterial species, to the best of our knowledge there have been no studies focusing on *T. lanceolata* against *Y. enterocolitica* growth. *T. lanceolata* berry and leaf extracts were prepared and their antibacterial potential was assessed against this pathogen.

MATERIALS AND METHODS

Plant collection and extraction

Seedless, semi-dried *T. lanceolata* berries and dried leaf materials were acquired from Go Wild Harvest, Australia and stored at -30°C. Extracts were prepared as previously described.[14-15] Briefly, plant materials were thawed and ground into a coarse powder. One-gram quantities of the ground plant materials were weighed into tubes in triplicate, followed by the addition of 50 mL of water, methanol, hexane, chloroform or ethyl acetate respectively. All solvents were obtained from Ajax Fine Chemicals, Australia (AR grade). The berry and leaf materials were extracted in each solvent for 24 hr at 4°C with gentle shaking. The extracts were filtered through with filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The dried extracts were weighed and resuspended in 10 mL deionised water (containing 1% DMSO).

Qualitative phytochemical studies

Phytochemical analyses of the extracts for the presence of alkaloids, flavonoids, phenolic compounds, polysteroids, anthraquinones, cardiac glycosides, saponins, tannins and triterpenoids were conducted as previously described.[16,17]

Antioxidant capacity

The antioxidant capacity of each extract was assessed using the DPPH free radical scavenging method with modifications.[18,19] Briefly, ascorbic acid references (0-25 µg per well) were prepared and the absorbances recorded at 515 nm. All assays were completed alongside controls on each plate and all tests performed three times in triplicate (n=9). The antioxidant capacity (based off of DPPH free radical scavenging ability) was calculated for each extract and expressed as µg ascorbic acid equivalents per gram of the original plant material extracted.

Clinical *Yersinia enterocolitica* strain

The clinical isolate strain of *Yersinia enterocolitica* used in this study was supplied by Ms. Jane Gifkins of the School of Natural Sciences Griffith University, Australia. Confirmation of bacterial identity was confirmed as previously described.[20,21] All growth studies were performed using nutrient agar (Oxoid Ltd., Australia) under aerobic conditions. Incubations were at 30°C and the bacterium was subcultured and maintained in nutrient broth at 4°C. Subculture purity was periodically confirmed as previously described.[22,23]

Evaluation of antimicrobial activity

The antimicrobial activity of the *T. lanceolata* extracts were determined using a modified disc diffusion assay.[24] Briefly, 100 µL of *Y. enterocolitica* was grown in 10 mL of fresh nutrient broth until they reached a count of ~10^8 cells/mL. Aliquots (100 µL) of the bacterial suspensions were spread onto plates and each extract was tested for antibacterial activity using 6 mm sterilised filter paper discs. Discs were infused with 10 µL of *T. ferdinandiana* extracts, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4°C for 2 h before incubation at 30°C for 24 hr. The diameters of the inhibition zones were measured to the closest whole millimetre. Each assay was performed three times, with internal triplicates (n=9). Mean values (± SEM) are reported in this study. Standard discs of chloramphenicol (2 µg) were prepared and used as positive controls to compare antibacterial activity. Filter discs infused with 10 µL of distilled water were used as a negative control.

Minimum inhibitory concentration (MIC) determination

The Minimum Inhibitory Concentrations (MIC) of the extracts was determined as previously described.[25] Briefly, the plant extracts were diluted in deionised water and tested across a range of concentrations. Discs were infused with 10 µL of the extract dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above and graphs of the zone of inhibition versus concentration were plotted. Linear regression was used to determine MIC values.

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared using 0.22 µM filter-sterilised, distilled water (4 mg/mL) and serially diluted in synthesised, artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii mortality assay.[26] Briefly, 400 µL of seawater containing ~43 (mean 43.2, n = 155, SD 14.5) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used in the bioassay. Volumes of 400 µL of reference toxin or the diluted plant extracts were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A 400 µL volume of seawater was used as a negative control and was run in triplicate for each plate. All treatments were performed three times, each with internal triplicates (n=9). The wells were checked at regular intervals and the deceased nauplii were counted. Nauplii death was determined if no appendage movement was observed within a 10 sec interval (or any period thereafter). 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 calculated using probit analysis.

Statistical analysis

Data is expressed as the mean ± SEM of three independent experiments, each with technical triplicates (n=9).

RESULTS

Extraction of 1 g of the *T. lanceolata* plant materials with the solvents yielded dried plant extracts ranging from 9 mg (*T. lanceolata* leaf hexane extract) to 144 mg (methanolic *T. lanceolata* leaf extract; Table 1). Aqueous and methanolic extracts generally gave relatively high yields of dried extracted material compared to the other extracts. The dried extracts were resuspended in 10 mL of deionised water (containing 1 % DMSO), resulting in the extract concentrations shown in Table 1.

Qualitative phytochemical studies showed little difference between the aqueous and methanolic extracts. However, there were notable differences between these and the ethyl acetate extracts. High levels of phenolics (both water soluble and insoluble) were extracted in the aqueous and methanolic samples. There were substantially lower levels detected in the corresponding ethyl acetate extracts. Similarly, there was a lower level of flavonoids detected in the ethyl acetate extracts than the corresponding aqueous and methanolic extracts. Triterpenes were detected in both methanolic and ethyl acetate extracts, although they were absent in the aqueous extracts.

To assess the inhibitory activity of the crude *T. lanceolata* plant extracts against *Y. enterocolitica*, 10 µL aliquots of each were screened with a disc diffusion assay. Antibacterial activity against *Y. enterocolitica* was observed in all 10 extracts tested (Figure 1). The methanolic berry extract was the most potent inhibitor of growth, with inhibition zones of 11.3 ± 0.3 mm. Similarly, strong inhibitory zones were observed in ethyl

Table 1: The mass of dried extracted material, the concentrations after resuspension in deionised water, qualitative phytochemical screenings and antioxidant capacities of the *T. lanceolata* leaf and berry extracts.

Extract	Mass of Dried Extract (mg)	Concentration of Resuspended Extract (mg/mL)	Total Phenolics	Water Soluble Phenolics	Water Insoluble Phenolics	Cardiac Glycosides	Saponins	Triterpenes	Polysteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
TLLW	134	13.4	+++	+++	+++	–	++	–	–	–	–	+++	–	–	–
TLLM	144	14.4	+++	+++	+++	–	+++	+	–	–	–	+++	–	–	–
TLLC	37	3.7	+	–	+	–	–	–	–	–	–	–	–	–	–
TLLH	9	0.9	–	–	–	–	–	–	–	–	–	–	–	–	–
TLLE	17	1.7	+	+	++	–	–	+	–	–	–	++	–	–	–
TLBW	111	11.1	+++	+++	+++	–	–	–	–	–	–	+++	–	–	–
TLBM	171	17.1	+++	+++	+++	–	++	+	–	–	–	+++	–	–	–
TLBC	47.2	4.7	+	+	+	–	–	–	–	–	–	–	–	–	–
TLBH	11	1.1	–	–	–	–	–	–	–	–	–	–	–	–	–
TLBE	56.7	5.7	+	+	++	–	+	++	–	–	–	++	–	–	–

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. TLLW = aqueous *T. lanceolata* leaf extract; TLLM = methanolic *T. lanceolata* leaf extract; TLBC = chloroform *T. lanceolata* leaf extract; TLBH = *T. lanceolata* leaf extract TLLE = ethyl acetate *T. lanceolata* leaf extract; TLBW = aqueous *T. lanceolata* berry extract; TLBM = methanolic *T. lanceolata* berry extract; TLBC = chloroform *T. lanceolata* berry extract; TLBH = *T. lanceolata* berry extract; TLBE = ethyl acetate *T. lanceolata* berry extract.

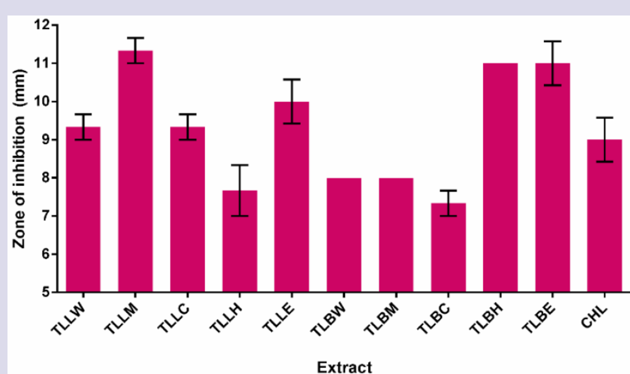

Figure 1: Growth inhibitory activity of *T. lanceolata* leaf and berry extracts against the *Y. enterocolitica* clinical isolate measured as zones of inhibition (mm). TLLW = aqueous *T. lanceolata* leaf extract; TLLM = methanolic *T. lanceolata* leaf extract; TLBC = chloroform *T. lanceolata* leaf extract; TLBH = *T. lanceolata* leaf extract TLLE = ethyl acetate *T. lanceolata* leaf extract; TLBW = aqueous *T. lanceolata* berry extract; TLBM = methanolic *T. lanceolata* berry extract; TLBC = chloroform *T. lanceolata* berry extract; TLBH = *T. lanceolata* berry extract; TLBE = ethyl acetate *T. lanceolata* berry extract; CHL = Chloramphenicol (2 μg). Results are expressed as mean zones of inhibition ± SEM.

acetate leaf extract (10.0 ± 0.6 mm) as well as the hexane (11.0 ± 0.0 mm) and ethyl acetate (11.0 ± 0.6 mm) berry extracts. The chloramphenicol control had inhibitory zones of 9.0 ± 0.6 mm which is notably less than several of the *T. lanceolata* extracts.

The antimicrobial efficacy was further quantified by determining the MIC values (Table 2). All the extracts were determined to be potent inhibitors of *Y. enterocolitica* growth, with MIC <1000 μg/mL for all extracts except the chloroform berry extract. The *T. lanceolata* leaf extracts were generally more potent *Y. enterocolitica* growth inhibitors than the corresponding berry extracts. Indeed, a MIC of 30 μg/mL was determined for the ethyl acetate *T. lanceolata* leaf extract.

All extracts were initially screened at 2000 μg/mL in the *Artemia* nauplii assay as a measure of toxicity (Figure 2). For comparison, the reference toxin potassium dichromate (1000 μg/mL) was also tested in the bioassay. The potassium dichromate reference toxin was rapid in its onset, promoting nauplii death within the first 3 hr of exposure, with 100 % mortality evident following 4-5 hr (unpublished results). Similarly, all the *T. lanceolata* extracts displayed significant mortality rates following 24 hr exposure (>50%).

DISCUSSION

Despite the initial efficacy of clinically used antibiotics, recent increases in bacterial resistance have made the development of new antibiotic

Table 2: Minimum inhibitory concentration (µg/mL) of the plant extracts and LC_{50} values (µg/mL) in the *Artemia nauplii* bioassay.

Extract / Control	MIC	LC
TLLW	370	2665
TLLM	318	2096
TLLC	53	2540
TLLH	900	3058
TLLE	30	2766
TLBW	600	2376
TLBM	425	2573
TLBC	4700	1846
TLBH	34	2875
TLBE	276	3132
PD	-	186
SW	-	-

Numbers indicate the mean MIC and LC_{50} values of triplicate determinations. - indicates no inhibition.

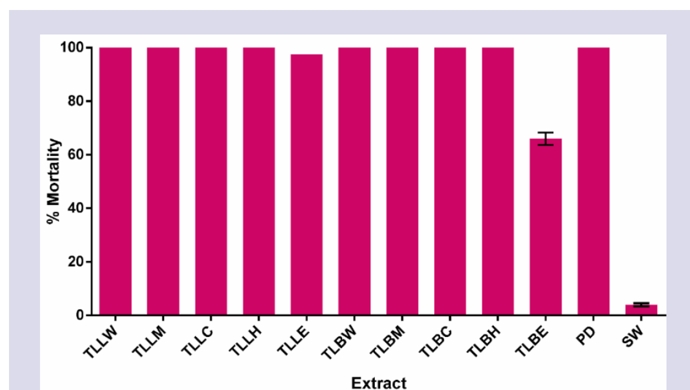

Figure 2: The lethality of the *T. lanceolata* extracts (2000 µg/mL) and the potassium dichromate control (1000 µg/mL) towards *Artemia franciscana* nauplii after 24 hr exposure. TLLW = aqueous *T. lanceolata* leaf extract; TLLM = methanolic *T. lanceolata* leaf extract; TLBC = chloroform *T. lanceolata* leaf extract; TLBH = *T. lanceolata* leaf extract TLLE = ethyl acetate *T. lanceolata* leaf extract; TLBW = aqueous *T. lanceolata* berry extract; TLBM = methanolic *T. lanceolata* berry extract; TLBC = chloroform *T. lanceolata* berry extract; TLBH = *T. lanceolata* berry extract; TLBE = ethyl acetate *T. lanceolata* berry extract; PD = potassium dichromate control; SW = artificial seawater control. Results are expressed as mean % mortality ± SEM.

therapies a high priority.[27] A parallel decrease in the introduction of new antibiotic therapies in recent years has further compounded this problem. As a result, interest in re-evaluating medicinal plants for new antibiotic chemotherapies has escalated substantially in recent years.[28] Our study was undertaken to investigate the potential of the endemic Australian plant *T. lanceolata* to inhibit the growth of *Y. enterocolitica*, thereby blocking yersiniosis and treating it once it is established. *T. lanceolata* was selected for this study as it has a history of therapeutic usage to treat microbial infections,[1] and numerous recent investigations have reported on its antibacterial properties against a broad panel of bacterial pathogens, including several bacteria associated with diarrhoea and dysentery.[4,6,7] *T. lanceolata* extracts have also recently been reported to inhibit the proliferation of the gastrointestinal protozoan parasite *Giardia duodenalis*,[7] indicating its therapeutic potential against both prokaryotic and eukaryotic pathogens. However, despite the relative wealth of information into the therapeutic potential of *T. lanceolata*, it is yet to be comprehensively studied for antibacterial activity against many bacterial species associated with food poisoning. Here we report growth inhibitory activity for *T. lanceolata* berry and leaf extracts against the bacterial cause of the gastrointestinal disease yersiniosis; an acute infection acquired through the ingestion of food (particularly pork)[12] contaminated with *Y. enterocolitica*.

Potent *Y. enterocolitica* growth inhibitory activity was evident in the *T. lanceolata* fruit and leaf extracts. Indeed, with the exception of the chloroform berry extract, MIC values of substantially <1000µg/mL were calculated for all extracts. The ethyl acetate and chloroform leaf extracts (MICs of 30 and 53 µg/mL respectively) and the hexane berry extract (MIC = 34 µg/mL) were particularly potent growth inhibitors. The berry and leaf methanolic and water extracts, as well as the ethyl acetate leaf extract, also exhibited strong growth inhibitory activity against *Y. enterocolitica*; albeit with higher MIC values (250-300µg/mL). Therefore, these extracts show good potential in the prevention and treatment of yersiniosis and are potential targets for future drug discovery. Furthermore, given that *T. lanceolata* berries and leaves are edible herbs and spices, their addition to foods would not only provide pleasant flavour enhancements, but could also inhibit food spoilage and the chances of contracting food poisoning (including yersiniosis).

Detailed analyses into the phytochemistry of the *T. lanceolata* fruit and leaf extracts was beyond the scope of this study. However, several notable compounds have been previously been reported in *T. lanceolata* extracts and essential oils.[1] These include multiple monoterpenoids (e.g. 1, 8-cineole, terpinen-4-ol, α-pinene and β-pinene) and sesquiterpenoids (particularly polygodial), flavonoids (including quercetin and rutin), other phenolics (including coumaric acid and caffeic acid) and hydrocarbons. Many of these compounds have also been isolated from other plant species and have been shown to have potent antimicrobial activity.[1] Therefore, these components may also contribute to the *Y. enterocolitica* growth inhibitory properties of the extracts tested in this study. Of particular note, relatively high abundances of the sesquiterpenoid polygodial have been reported in *T. lanceolata* berry and leaf extracts, with higher relative levels detected in the berry extracts. Indeed, polygodial can account for approximately 40% of commercial *T. lanceolata* essential oil components.[1,29] Interestingly, several studies have reported the therapeutic properties of this compound, including its antibacterial,[29] antifungal,[30] anti-hyperalgesia,[31] anti-inflammatory, antiallergic and vasorelaxation activities.[32] Other structurally similar sesquiterpenoids have also been reported in similar *T. lanceolata* extracts.[7] Of particular interest, salidroside was present in all inhibitory *T. lanceolata* berry extracts. Salidroside has been linked with antibacterial and anti-parasitic activity in multiple plants. Plants of the genus *Warburgia* are known to have significant levels of salidroside[33] and several *Warburgia* spp. have been reported to have strong antimicrobial and antiparasitic activities.[34,35]

T. lanceolata berry and leaf extracts have also been reported to contain an abundance of gallotannin components.[7] Gallotannins have been reported to inhibit the growth of a broad spectrum of microbial species[36] via binding cell surface lipoteichoic acid and proline-rich cell surface proteins,[37,38] and by inhibiting glucosyltransferase enzymes.[39] The stilbene combretastatin A1 was also putatively identified in all *T. lanceolata* berry extracts,[7] although the relatively low peak size indicates that it is present in low abundance. Combretastatin's are well known for their potent ability to block cancer cell progression and induce apoptosis by binding intracellular tubulin, thereby disrupting microtubule formation.[40] Combretastatin's act in a similar fashion to that of colchicine (N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptlen-7-yl]

acetamide) by binding the colchicine's binding site in tubulin.[41] Thus, the *T. lanceolata* combretastatin's may block bacterial replication events.

The findings reported here also indicate that the *T. lanceolata* berry and leaf extracts were nontoxic, with LC_{50} values substantially >2000 μg/mL. Toxicity was assessed in this study using the test organism *A. franciscana*. The lack of toxicity of the *T. lanceolata* extracts in our study indicates that the extracts are safe for medicinal usage. This is hardly surprising as *T. lanceolata* is highly nutritious and has long been used as a spice (berries) and herb (leaves). However, whilst the extracts examined in this report have potential as *Y. enterocolitica* growth inhibitory agents, caution is needed before these compounds can be applied to medicinal purposes. Toxicity towards *A. franciscana* has previously been shown to correlate well with toxicity towards human cells for many toxins.[7] However, further studies are required to determine whether this is also true for the *T. lanceolata* extracts examined in these studies. The results of this study indicate that the *T. lanceolata* extracts warrant further study due to their *Y. enterocolitica* growth inhibitory activity. Purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents.

CONCLUSION

The results of this study demonstrate the potential of *T. lanceolata* berry and leaf extracts to inhibit *Y. enterocolitica* growth. The mid-low polarity ethyl acetate, chloroform and hexane *T. lanceolata* extracts were particularly potent growth inhibitors, with MIC values < 100 μg/mL quantified. However, before being deemed acceptable for therapeutic uses, further cell line toxicity studies are required to verify the safety of these extracts. Furthermore, studies aimed at the purification and identification of the bioactive components are required to examine the mechanisms of action of these extracts.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

ABBREVIATIONS

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

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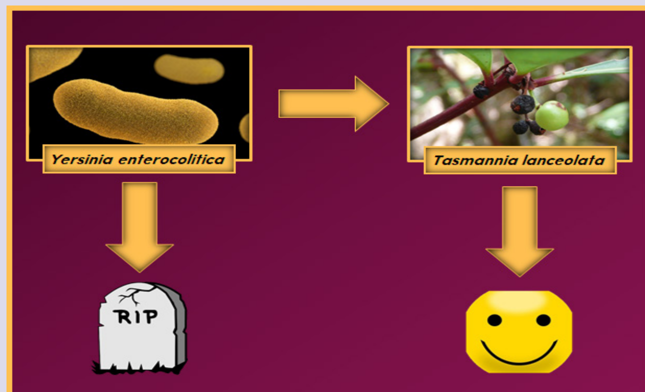
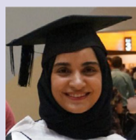
PICTORIAL ABSTRACT



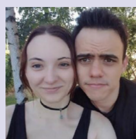
SUMMARY

- *T. lanceolata* berry and leaf extracts were screen for growth inhibitory activity against Y. entercolitica.
- Most extracts were potent inhibitors of Y. entercolitica growth
- The leaf extracts were generally more potent than the corresponding berry extracts.
- The leaf ethyl acetate and chloroform (30 and 53 ug/mL respectively) and the berry hexane extract (34ug/mL) were particularly potent.
- All extracts were nontoxic in the Artemia nauplii assay.

ABOUT AUTHORS



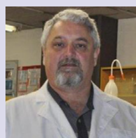
Ms. Huda Aldosary is undertaking her Ph.D at Griffith University (Brisbane, Australia) under the supervision of Dr. Anthony Greene. Her primarily interests involve the investigation of bacterial hydrocarbon degradation and the potential for these processes in the remediation of contaminated sites. She has extensive experience with anaerobic organisms and specialises in extremophiles.



Dr. Mitchell Henry Wright is a Geomicrobiologist who received his Ph.D in 2014 for his work investigating the manganese reduction/oxidation characteristics of environmental bacteria. From 2016 to 2018 he undertook a postdoctoral researcher role under the mentorship of Prof. Bradley Tebo, where he explored the bacterial oxidative formation and removal of complexed Mn (III) and the implications of these processes on the global ocean. Upon returning to Australia, Dr. Mitchell H. Wright was recruited by First Choice College and to date, oversees their Department of Research and Development. Additionally, he has returned to his former lab (lead by Dr. Ian Cock) to continue his research into the antimicrobial potential of native plants.

Mr. Cameron Lee completed his Bachelor of Science (BSc) in 2015 and is currently undertaking his Ph. D with Dr. Anthony Carlson Greene. His research involves the investigation of thermophilic anaerobes that utilize toxic metals in anaerobic respiration (including uranium and arsenic). He has extensive experience in anaerobic cultivation/isolation and in numerous analytical techniques associated with heavy metal analysis.

Dr. Anthony Carlson Greene is a senior lecturer and researcher at Griffith University, Brisbane, Australia. He obtained his Ph.D in Microbiology from the University of New South Wales and focuses on extreme environments, Bioremediation and Geomicrobiology. His specific interests include the microbial ecology of thermophilic, saline and alkaliphilic environments and the mechanisms and industrial potential of extremophilic bacteria contained therein.



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 over 100 publications across a variety of peer reviewed journals.