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Original Article

Comparison of the Antibacterial Activities of *Terminalia ferdinandiana* Exell. Growing in Geographically Distinct Regions of Australia

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ABSTRACT

Background: *Terminalia ferdinandiana* Exell. fruit have been used by the First Australians as a nutritious food and as a medicine for thousands of years. The antibacterial properties of *T. ferdinandiana* fruit extracts are well reported. However, the therapeutic potential of plants growing in different locations and environmental conditions have not previously been compared. **Aim:** This study compares the antibacterial efficacy of fruit harvested from two distinct locations in Australia and correlates these activities with their physiochemical properties. **Materials and Methods:** The growth inhibitory activity of the *T. ferdinandiana* fruit extracts were evaluated using solid phase disc diffusion and liquid microdilution MIC assays. *Artemia* nauplii bioassays were used to screen and compare the extracts from both locations. **Results:** The *T. ferdinandiana* extracts prepared from fruits sourced from the two distinct regions of Australia inhibited the growth of the panel of bacteria screened, including a highly antibiotic MRSA strain. In general, the methanolic extracts were substantially better inhibitors of bacterial growth than the aqueous extracts, and extracts prepared using the Northern Territory (NT)-derived fruit were substantially more potent than the Western Australian (WA) fruit extracts. MICs substantially <1000 µg/mL were noted for the NT methanolic fruit extract against the reference *S. aureus* and MRSA bacterial strains respectively. The potency of this extract against the MRSA strain indicates that this extract may function via a distinct mechanism compared to the standard antibiotics tested. Interestingly, the greater antibacterial potency of the NT fruit extracts correlated to high ascorbic acid levels, indicating that the antibacterial mechanism may involve modulation of the redox state. All extracts were nontoxic in the *Artemia* nauplii toxicity assay, indicating their safety for therapeutic usage. **Conclusion:** The *T. ferdinandiana* fruit extracts prepared from both NT and WA each had noteworthy antibacterial activity, although greater activity was noted for the NT fruit extracts. This activity correlated with the antioxidant/ascorbic acid content of the extracts. All extracts were also nontoxic in the *Artemia* nauplii bioassay, although future studies using mammalian cell lines are required to confirm their safety for therapeutic use.

Keywords: Kakadu plum, Combretaceae, Antibiotic-resistance, MRSA, Redox modulation, Ascorbic acid.

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INTRODUCTION

Antibiotics have drastically transformed modern medicine by increasing life expectancy and the quality of life. However, the evolution of multi-drug resistant (MDR) bacteria has led to a rise of deadly pathogens with reduced sensitivity towards many different classes of antibiotics. There is an urgent need to develop new antibiotic therapies to combat the rising tide of bacterial antibiotic resistance.[1] Many of the current clinically available antibiotics, most of which were developed before the 21st century, are now ineffective or of substantially reduced efficacy.[2] Thus, the discovery of new bioactive compounds that kill or suppress the growth of bacterial pathogens is amongst the most urgent problems currently facing medical science.

Extensive efforts are underway to search for plant compounds as novel sources of new antibacterial therapies. These molecules may themselves possess antibacterial properties, or they may potentiate the efficacy of conventional antibiotics to treat MDR bacterial infections.[3] Plants native to Australia have documented antimicrobial activities.[4] *Terminalia ferdinandiana* Exell. (Kakadu

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Plum) is a plant native to the Northern Territory (NT) and Western Australian (WA) regions of the country. It is a small to moderate-sized, semi-deciduous tree that grows 4-10 m in height. It produces pale green fruit, cream flowers, and large oval leaves.[5] It has been traditionally used by the Australian First Peoples for its antimicrobial properties in treating skin conditions and may be consumed as a tea for respiratory infections, or to boost energy and immunity before hunting.[6]

High levels of antioxidants and therapeutic phytochemicals are found within *Terminalia* spp.[7] Examples of these compounds include tannins, ellagic acid, chebulic acid, castalagin and chebulagic acid. Interestingly, some of these compounds are potent inhibitors of bacterial growth, which has been associated with their strong antioxidant activities.[8] *Terminalia ferdinandiana* Exell. also displays high levels of ascorbic acid, as well as high levels of other antioxidants, which has garnered recent attention in the current market for supplements to treat multiple chronic diseases including rheumatoid arthritis, Alzheimer's disease, cancer, cardiovascular disease, and diabetes, as well as against acute pathogenic infections.[9]

Notably, most of the studies that have screened *T. ferdinandiana* extracts for the ability to treat antibiotic bacterial infections have focussed on plants from a single area (most frequently, coastal areas of the Kakadu and Arnhem Land areas of the NT, Australia). Plant materials derived from other regions of Australia have been largely neglected. *Terminalia ferdinandiana* can also grow further inland (in areas with lower rainfall) and in large areas of northern WA. The location in which plants grow affects the availability of nutrients and minerals, as well as climatic conditions in which they grow (e.g. rainfall, heat, humidity, seasonality). These conditions may profoundly influence their metabolic activity, and therefore the phytochemical profile. Differing phytochemical profiles may subsequently affect the bioactivities and potency of the fruit of this species. These differences are yet to be evaluated for *T. ferdinandiana* fruit derived from different locations. In the present study, *T. ferdinandiana* fruit were sourced from two distinct regions of Australia. The "Northern Territory (NT)" derived fruit were sourced from the Kakadu region of the NT, within 50 km of the coast, whereas the "Western Australia (WA)" fruit were sourced from the semi-arid Kimberley region of northwestern Australia. Fruit extracts from both regions were evaluated and their activity and their physiochemical properties were compared to highlight difference between plants sourced from these distinct environments.

MATERIALS AND METHODS

Plant sources

Northern Territory *T. ferdinandiana* fruit were provided by David Boehme of NT Wild Harvest as frozen whole fruit. The fruit was verified by David Boehme and were originally sourced from Kakadu National Park, under government collection permits. The fruit was thawed, the seed was removed, and the plant flesh was dried in a Sunbeam™ food dehydrator. The dried fruit was then powdered using a coffee grinder and stored at in an airtight container at -30°C until use. Western Australian *T. ferdinandiana* fruit were supplied and verified by Jacinta Monck of Kimberley Wild Gubinge as dried and ground fruit and were originally sourced from the Dampier Peninsula, WA. The powder was stored at -30°C until use.

Plant extractions

One-gram quantities of each plant were weighed into separate tubes and either distilled water or AR grade methanol (Ajax Fine Chemicals, Australia) were added to give a total volume of 50 mL. The tubes were mixed for 20-24 hr and the particulate matter was removed from each sample by filtration through Whatman number 54 filter paper. The filtrates were air dried by evaporation and the crude extracts weighed to determine extraction yield and resuspended in 10 mL 1% dimethyl sulfoxide (DMSO) (AR grade, Ajax Fine Chemicals, Australia). The resuspended extracts were then sonicated, and the solutions sterilised via passage through 0.2 µm filters (Millipore, Australia). The final extracts were stored as 1 mL aliquots at -20°C until required.

Bacterial cultures

The bacterial species used in this study were *Staphylococcus aureus* (ATCC# 25923), MRSA (ATCC# 43300), *Staphylococcus epidermidis* (ATCC# 122292) and *Streptococcus pyogenes* (ATCC# 12384). All bacteria were maintained on Mueller-Hinton (MH) agar plates, and in MH broth (Oxoid Ltd., Australia). Agar supplemented with 2% NaCl were used for *S. aureus* strains to further ensure their purity.

Disc diffusion assays

Extracts were initially assessed for their antibacterial activities on MH agar.[10] Plates were covered with 100 µL of a 0.5 McFarland bacterial preparation in MH broth, and 6 mm filter paper discs were affixed to the plates using sterile forceps. Volumes of 10 µL of each extract were applied to separate discs, with an equivalent volume of 1% DMSO tested in parallel as control discs. Agar plates were incubated for 18-20 hr at 35°C, and inhibition measured as a zone of inhibition (ZOI) surrounding the disc measured to the nearest whole millimetre. Samples failing to inhibit bacterial growth were recorded as the diameter of the disc (6 mm).

Liquid dilution assays

A standard 96-well microplate method[8] was used to determine minimum inhibitory concentration (MIC) values for all extracts and antibiotics. A volume of 100 µL of MH broth was added to each well of the plate, followed by addition of 100 µL of each individual sample to the top row of the plates. The solutions were mixed and 100 µL was withdrawn and added to the second row, and thoroughly mixed. This dilution method was repeated

down each column. A volume of 100 µL was aspirated from the wells of the final row and discarded following mixing. A 1:100 dilution of the 0.5 McFarland bacterial solution was prepared in MH broth, and 100 µL was added to each well on the plate. Following incubation of each plate for 20-24 hr at 35°C, 40 µL of 0.04% ρ-iodonitrotetrazolium chloride was added to each well and re-incubated for 4 hr. Bacterial growth was evidenced by the production of red-pink dye within the wells. MIC values (µg/mL) were measured as the lowest concentration of extract or antibiotic that failed to generate optically visible red-pink colour. Extract MIC values >5000 µg/mL were considered inactive; MIC values between 2000 and 5000 µg/mL were considered low activity; 1000–2000 µg/mL were considered moderate activity; 400–1000 µg/mL were considered noteworthy activity; 100–400 µg/mL were considered good activity; and <100 µg/mL were considered high activity.[11]

Qualitative phytochemical assays

Qualitative evaluations of the presence and relative abundances of the tannins, saponins, total phenolics, water soluble phenols, cardiac glycosides, triterpenoids, phytosterols, alkaloids, flavonoids and anthraquinones in each extract were tested using standard protocols.[12]

Artemia nauplii toxicity assays

All *T. ferdinandiana* extracts were screened for toxicities using the *Artemia franciscana* Kellogg nauplii lethality assay method.[13] Briefly, the eggs of *A. franciscana* (Aquabuy, Auburn Australia) were hatched and grown at 25°C in artificial seawater (Red Sea Pharm Ltd., Israel). Briefly, 400 µL of each plant extract was added to individual wells of a 48-well plate, and 400 µL of artificial seawater containing 40-60 hatched eggs was added to each well. Wells containing artificial seawater served as negative controls, whilst additional wells containing a 4 mg/mL solution of potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) were included on each plate as a positive control/reference toxin. The plates were incubated for 24 hr at approximately 25°C. Toxicity levels of the extracts were determined by calculating the concentration of extract required to cause 50% lethality of *A. franciscana*, represented as LC_{50} values, using probit analysis.

Government laboratories chemical analysis

Each dried fruit powder was supplied to the Australian Government National Measurement Institute (Port Melbourne, Australia) for standardised analysis of microbial contaminants, proximate analysis, as well as quantification of trace elements, vitamins and fatty acid contents. Analysis of the NT fruit (job number WILD08/110509) was performed by Glenda Scott (microbiology), Paul Adorno (inorganics), Dr Nahar Syeda and Paul Adorno (food composition), and Samantha Duong (organics analysis). Analysis of the WA fruit (job number KIMB10/160229) was performed by Glenda Scott (microbiology), Nunzio Limongiello and Devika Kodituwakku (inorganics), Norbert Strobel, Paul Adorno, Munifa Puente and Leo Demel (food composition).

Statistical analysis

Statistical analysis was conducted with data expressed as the mean ± SEM of three independent experiments. To calculate the statistical significance, bar graphs with error bars were used for each bacterial species, with p values ≤0.05 considered statistically significant, and a p value ≤ 0.01 regarded as highly statistically significant. All extracts were tested in triplicate in disc diffusion and liquid dilution assays. Antibiotics were tested in triplicate for disc diffusion assays, and in duplicate for liquid dilution assays to ensure the reproducibility of the results.

It was not possible to perform statistical analysis on the broth microdilution assays. However, the reliability of MIC values was ensured by repeating the broth microdilution assays twice on separate days. In addition, two replicates per assay (n = 4) were conducted. These measures were taken to confirm that the results were reproducible for all extracts, antibiotics and combinations tested.

RESULTS

Extract yields and qualitative analysis of phytochemical classes

The concentrations of each extract, along with a qualitative determination of the various phytochemical classes present are shown in Table 1. The yields across all four extracts were similar and in the range of 36-49 mg/mL. All extracts were rich in water soluble and insoluble phenols, as well as tannins and triterpenoids. Cardiac glycosides, anthraquinones, phytosterols and alkaloids were not detected in any of the extracts. Interestingly, flavonoids were not detected in the WA extracts, although they were present in high abundances in the NT extracts. Additionally, a moderate level of saponins was detected in all extracts except for the WA methanolic fruit extract.

Disc diffusion assays

The inhibition of bacterial growth on agar by extracts and reference antibiotics is shown in Figure 1. All extracts inhibited the growth of *S. aureus*, although the NT extracts were more effective than the WA samples. The NT extracts were also substantially more effective against MRSA, whereas the WA extracts were unable to inhibit the growth of that pathogen. The aqueous and methanolic extracts for the NT plant were active against *S. pyogenes* and *S. epidermidis*. However, only the methanolic WA extract was active against these bacteria in this assay, as the aqueous extract failed to show any activity on agar.

Table 1: Concentrations of the plant extracts and their qualitative phytochemical analysis.

Extract type	Yield[a]	Phytochemical class and abundances									
		Water Soluble phenols	Water Insoluble phenols	Cardiac Glycosides	Saponins	Triterpenoids	Phytosterols	Alkaloids	Flavonoids	Tannins	Anthraquinones
NT KP-Aq	36.7	+++	++	-	++	++	-	-	+++	+++	-
NT KP-MeOH	48.7	+++	++	-	++	+	-	-	+++	+++	-
WA KP-Aq	40.9	+++	+++	-	++	+++	-	-	-	+++	-
WA KP-MeOH	42	+++	+++	-	-	+++	-	-	-	+++	+

[a]Values obtained following resuspension in 1% DMSO. +++ indicates a large response in the assay, ++ indicates a moderate response, + indicates a minor response, - indicates no response. KP = Kakadu plum (*T. ferdinandiana*), NT = Northern Territory, WA = Western Australia, Aq = aqueous and MeOH = methanol.

MIC determination by liquid dilution analysis

Table 2 shows the MIC values for the plant extracts and for the reference antibiotics, as determined by microdilution broth assays. The NT extracts inhibited the growth of all bacterial species in the microdilution broth cultures. Low activities were observed for the aqueous extracts. The methanolic extracts showed good activities against *S. aureus* and MRSA, whilst moderate activities were recorded against *S. pyogenes* and *S. epidermidis*. Interestingly, the activity of the methanolic NT extracts were higher for MRSA than for the susceptible *S. aureus* species. Generally, the MIC values were substantially higher for the WA extracts against each of the bacteria tested than the corresponding NT extracts, indicative of the lower potency of the WA extracts in the microdilution broth assay.

Toxicity evaluations

All extracts were assayed for their effects against *Artemia* nauplii (brine shrimp larvae) as a measure of toxicity levels. None of the extracts produced an LC_{50} value >50% and all extracts were therefore all defined as nontoxic in this assay.

Analysis of the physical and chemical properties of the *T. ferdinandiana* plant materials

All extracts prepared from the plant material sourced from both the NT and WA regions of Australia inhibited the growth of several of the bacterial species tested, although the NT-derived plant material resulted in substantially better antibacterial activity than the WA-derived plant material. Therefore, samples of each plant material were sent to the Australian Government National Measurement Institute (Port Melbourne, Australia) to evaluate their physiochemical properties. A comparison of the NT and WA plant materials is shown in Table 3. As *T. ferdinandiana* fruit is renowned for its high antioxidant capacity, it was of particular interest to compare the levels of antioxidant vitamins, and antioxidant capacities of each plant material. Whilst the NT and WA-derived plant material both had very high yields of plant material, the NT *T. ferdinandiana* fruit had substantially higher overall antioxidant activity than the WA fruit, as determined by total ORAC assay (~17% greater). Whilst both plant materials contained negligible levels of α-tocopherol, there were substantial differences in ascorbic acid levels. Indeed, the NT-derived fruit had approximately double the amount of ascorbic acid than the WA fruit (16,000 compared with 8,500 mg/100g of dried fruit respectively). However, whilst this is a large difference, the levels of ascorbic acid in both fruit materials is very high, and may contribute to the biological activities reported herein. These differences in ascorbic acid content between the fruits may contribute to the greater potency of the NT *T. ferdinandiana* fruit, although this remains to be verified.

Several other trends were also noted. In particular, substantial differences were noted in the sodium contents of the two fruits,

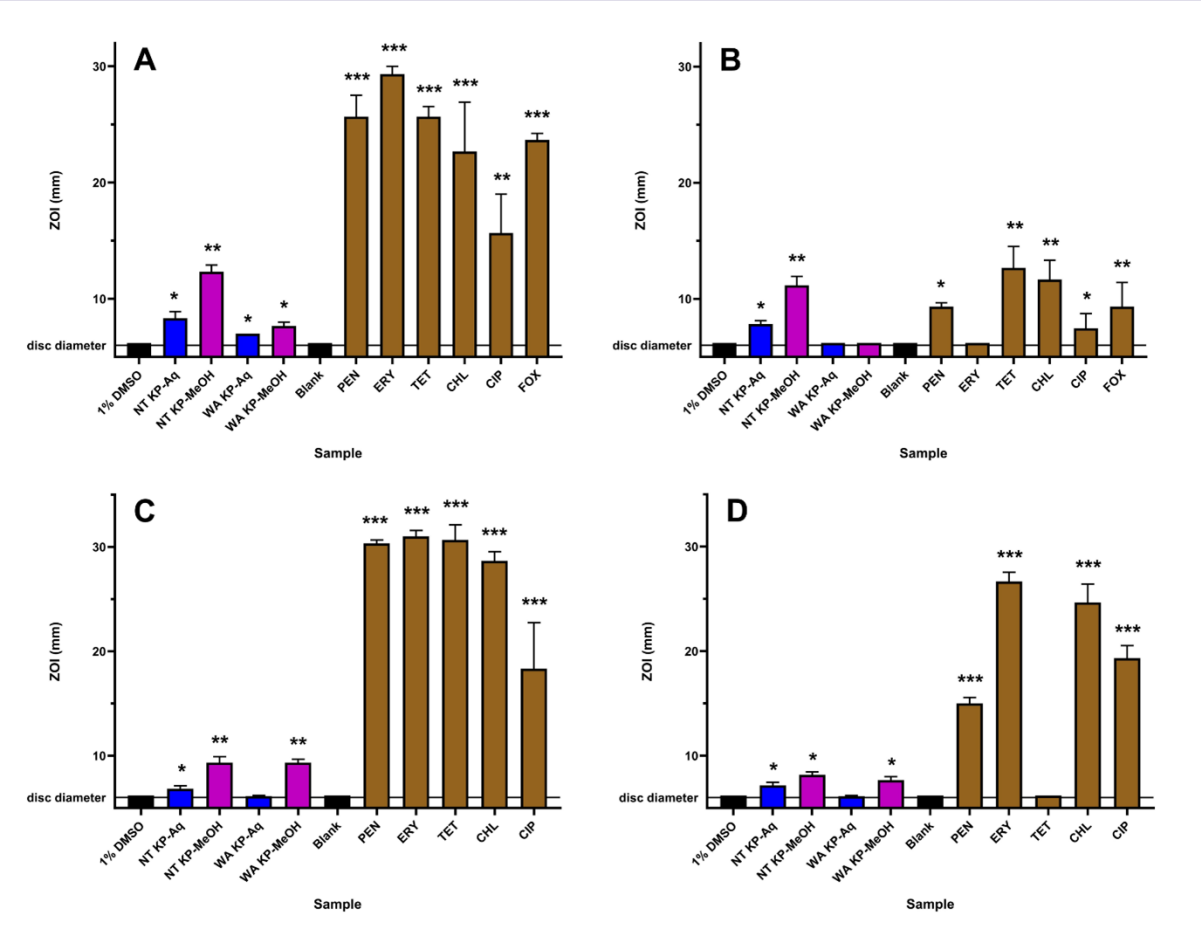

Figure 1: Agar disc diffusion assays for plant extracts and reference antibiotics against (A) *S. aureus*, (B) MRSA, (C) *S. pyogenes* and (D) *S. epidermidis*. The ZOI values were measured in mm and the disc sizes were 6 mm, as indicated on the y-axis. Negative control discs contained 1% DMSO (for crude extracts) or blank discs (for antibiotics) and are shown by the black bars. Aqueous and methanolic extracts are indicated in blue and purple bars, respectively, while antibiotics are shown as brown bars. Each value is expressed as mean ± SEM of triplicate assays of each extract and antibiotic. Results are significantly different to the negative control if $p < 0.05$ (*), highly statistically significant if $p < 0.01$ (**) and very highly statistically significant if $p < 0.001$ (***). NT = Northern Territory, WA = Western Australia, KP = Kakadu plum (*T. ferdinandiana*), Aq = aqueous, MeOH = methanol; PEN = penicillin, ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol, CIP = ciprofloxacin and FOX = cefoxitin.

Table 2: MIC values of plant extracts and reference antibiotics against the four bacterial strains tested in this study.

Plant extract or Antibiotic	Bacterial species			
	S. aureus	MRSA	*S. pyogenes*	*S. epidermidis*
NT KP-Aq	2200	2200	2650	2280
NT KP-MeOH	825	526	1260	1508
WA KP-Aq	3006	>5000	3006	>5000
WA KP-MeOH	1312	>5000	2625	1925
PEN	0.039	>2.5	0.002	>2.5
ERY	0.625	>2.5	0.002	0.313
TET	0.313	0.078	0.078	>2.5
CHL	>2.5	>2.5	0.625	2.5
CIP	0.625	0.625	0.156	0.156

KP = Kakadu plum (*T. ferdinandiana*), Aq = aqueous, MeOH = methanol, AB = antibiotics, PEN = penicillin, ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol and CIP = ciprofloxacin. Values of >2.5 for the antibiotics indicate a lack of growth inhibition at the highest concentration of antibiotic examined.

Table 3: Department of Primary Industries analysis reports of dried *T. ferdinandiana* fruit material from Kakadu National Park, Northern Territory, Australia, and Pender Bay, Dampier Peninsula, Western Australia.

Class of test	Compound	Units	Northen Territory plum	Kimberley WA plum
Microbial contaminants	Standard plate count	CFU/g	<150	<150
	Yeasts	CFU/g	<100	<100
	Moulds	CFU/g	<100	<100
Physical parameters	Moisture	(g/100 g dried fruit)	6.9	8.5
	Ash	(g/100 g dried fruit)	4	5.8
	Energy	kJ/100 g dried fruit	1570	1110
Trace elements	Sodium	(mg/100 g dried fruit)	37	80
Proteins		(g/100 g dried fruit)	5.2	3.9
Carbohydrates and sugars	Carbohydrates	(g/100 g dried fruit)	82	40
	Total sugars	(g/100 g dried fruit)	5.1	27
	Fructose	(g/100 g dried fruit)	2.2	13
	Glucose	(g/100 g dried fruit)	2	10
	Sucrose	(g/100 g dried fruit)	0.9	4.2
	Maltose	(g/100 g dried fruit)	<0.2	<0.2
	Lactose	(g/100 g dried fruit)	<0.2	<0.2
Vitamins	Ascorbic acid	(mg/100 g dried fruit)	16,000	8,500
	α-Tocopherol	(mg/100 g dried fruit)	<0.1	Not measured
	ORAC Vit E equiv (hydro)	µmol/kg	1,230,800	1052900
	ORAC Vit E equiv (lipo)	µmol/kg	2,600	1,500
	ORAC Vit E equiv (total)	µmol/kg	1233400	1054400
Fats (general)	Total fats (Monjonnier extraction)	(g/100 g dried fruit)	2.4	0.8
	Saturated fats	(g/100 g dried fruit)	0.4	0.3
	Mono trans fats	(g/100 g dried fruit)	<0.1	<0.1
	Monounsaturated fats	(g/100 g dried fruit)	0.5	<0.1
	Omega 3 fats	(g/100 g dried fruit)	<0.1	0.1
	Omega 6 fats	(g/100 g dried fruit)	1.3	0.3
	Poly trans fats	(g/100 g dried fruit)	<0.1	<0.1
	Polyunsaturated fats	(g/100 g dried fruit)	1.3	0.4
	Trans fats	(g/100 g dried fruit)	<0.1	<0.1
Saturated fats	Total saturated fats	%	17.6	35.3
	C4:0 Butyric	%	<0.1	<0.1
	C6:0 Caproic	%	<0.1	<0.1
	C8:0 Caprylic	%	<0.1	0.4
	C10: Capric	%	<0.1	0.3
	C12:0 Lauric	%	<0.1	2
	C14:0 Myristic	%	0.1	1.2
	C15:0 Pentadecanoic	%	<0.1	0.4
	C16:0 Palmitic	%	11.9	26.4
	C17:0 Margaric	%	<0.1	0.3
	C18:0 Stearic	%	4.5	2

Class of test	Compound	Units	Northen Territory plum	Kimberley WA plum
	C20:0 Arachidic	%	0.6	1
	C22:0 Behenic	%	0.2	0.8
	C24:0 Lignoceric	%	0.2	0.4
Mono-unsaturated fatty acids	Total mono-unsaturated	%	23.6	4.6
	C14:1 Myristoleic	%	<0.1	<0.1
	C16:1 Palmitoleic	%	0.2	0.9
	C17:1 Heptadecenoic	%	<0.1	<0.1
	C18:1 Oleic	%	23.2	3.3
	C20:1 Eicosenic	%	0.1	0.2
	C22:1 Docosenoic	%	<0.1	<0.1
	C24:1 Nervonic	%	<0.1	<0.1
Poly-unsaturated fatty acids	Total Polyunsaturated fatty acids	%	58.6	51.3
	Total mono-unsaturated fatty acids	%	<0.1	<0.1
	Total poly trans fatty acids	%	0.1	0.4
	P:M:S	Ratio	3.3:1.3:1	1.5:0.1:1
	C18:2w6 Linoleic	%	56.1	35.8
	C18:3w6 gamma-Linolenic	%	<0.1	<0.1
	C18:3w6 α-Linolenic	%	2.4	<0.1
	C20:2w6 Eicosadienoic	%	<0.1	<0.1
	C20:3w6 Eicosatrienoic	%	<0.1	<0.1
	C20:3w3 Eicosatrienoic	%	<0.1	<0.1
	C20:4w6 Arichidonic	%	<0.1	<0.1
	C20:5w3 Eicosapentaenoic	%	<0.1	<0.1
	C22:2w6 Docosadienoic	%	<0.1	<0.1
	Omega 3 fatty acids	%	2.5	15.4
	Omega 6 fatty acids		56.1	35.9
	C22:4w6 Docosatetraenoic	%	<0.1	<0.1
	C22:5w3 Docosapentaenoic	%	<0.1	<0.1
	C22:6w3 Docosahexaenoic	%	<0.1	<0.1

Analysis was performed at the Australian Government National Measure Institute, in Port Melbourne, Australia.
CFU = colony forming units; P:M:S = ratio of polyunsaturated to monounsaturated to saturated fatty acids.

with the WA fruit containing more than twice the sodium content compared with the NT fruit (37 and 80 mg/100 g dried fruit respectively). As high sodium consumption is linked with increased blood pressure,[14] the NT fruit may be considered healthier in this aspect. However, foods with <400 mg sodium per 100 g are generally considered safe for consumption and <100 mg/100 g is generally considered to contain 'low salt' or to be 'salt reduced'.[15] Therefore, by definition, both of these fruits contain low sodium contents. Furthermore, this study measured the sodium contents of dried fruit powders. As substantial mass is lost upon drying, the contents of the fresh fruit would be substantially lower than these levels. Both fruits are therefore considered to contain low/healthy sodium levels. Conversely, the sodium content of foods helps to retard microbial spoilage[16] and the presence of microbial contamination must also be considered in low salt foods. Notably, bacteria, mould and yeasts were not present in detectable levels in either fruit material.

With respect to other macronutrients, proteins were measured in similar levels in both fruits, whilst the total carbohydrate level was substantially higher in the NT fruit, compared to the WA fruit (82 g/100 g fruit powder compared to 40 g/100 g fruit respectively). Interestingly, the trend is reversed for the free sugar's fructose, glucose and sucrose, all of which were detected in ≥5 fold higher

levels in the WA fruit than in the NT-derived fruit. Substantial differences were also noted for the lipid contents of the two fruits, with the NT fruit containing approximately three times the total lipid contents than the WA fruit (2.4 compared with 0.8 g/100 g dried fruit). Notably, the WA fruit contained a substantially higher % of saturated fats compared with the NT fruit (35.3% and 17.6% of the total fats respectively). Conversely, a greater proportion of unsaturated fats (particularly monounsaturated fats) were present in the NT fruit compared to the WA fruit (23.6% compared to 4.6% respectively).

DISCUSSION

Terminalia ferdinandiana fruit has attracted considerable recent interest for its nutritional value, particularly due to its high antioxidant contents and the presence of numerous phytochemicals with medicinal properties.[7] Indeed, *T. ferdinandiana* fruit have been reported to contain ascorbic acid levels up to 6% of the fruit wet weight, which is approximately 900 times greater than the level in an equivalent mass of blueberries.[17,18] High antioxidant contents have been linked with numerous therapeutic properties and substantial recent studies have screened the beneficial effects of *T. ferdinandiana* fruit. In particular, dysregulation of the cellular redox state has been linked to several chronic diseases including cancer, inflammation and diabetes,[7] and antioxidant supplementation can partly mitigate these effects.[19] Notably, *T. ferdinandiana* fruit extracts have been reported to have good anticancer[20,21] and anti-inflammatory properties.[22] *Terminalia ferdinandiana* fruit extracts have also been reported to have potent anti-*Giardia duodenalis* activity and several noteworthy compounds were identified.[23] Subsequent studies determined that the high levels of ascorbic acid present in the extracts potentiate the activity of other components by maintaining a reducing environment.[24]

Additionally, a number of studies have examined the antibacterial properties of *T. ferdinandiana* fruit extracts against a broad spectrum of bacterial pathogens,[25-27] including several extensively resistant pathogens (XDR), and have reported broad spectrum potent antibacterial activity.[8] However, the earlier studies all screened *T. ferdinandiana* fruit derived from a single geo-location and grown in similar environmental conditions. None of those studies considered differences in phytochemical compositions (and therefore differences in therapeutic properties) for different cultivars, and/or plants growing in different locations and conditions.

The *T. ferdinandiana* extracts prepared from the NT and Western Australia-derived fruits each inhibited the growth of the panel of bacteria screened, although the NT-derived fruit were substantially more effective antibacterial agents than the WA fruit. Indeed, MIC values ≤825 and 526 µg/mL were recorded for the methanolic NT extract against both *S. aureus* strains. Interestingly, the extract was substantially more effective against the MRSA strain (526 µg/mL) than against the reference (antibiotic-susceptible) *S. aureus* strain (825 µg/mL). Notably, this MRSA strain has also previously been shown to be highly resistant to a wide variety of other antibiotic classes and therefore activity against this strain is particularly interesting.[8] The enhanced potency of this extract against the MRSA strain compared to the reference strain is promising and indicates that the antibacterial mechanism may be distinct from the mechanisms of the other antibiotics towards which this bacterium is resistant, although this remains to be verified. If this subsequently proves to be the case, this is particularly interesting as the extract may contain novel chemical scaffold(s), which would allow for the development of further semi-synthetic analogues. Further studies are required to fully evaluate the phytochemical complexity of this extract.

Alternatively, the extract may contain compounds that themselves are ineffective antibacterial agents, although that block bacterial resistance mechanisms, thereby potentiating the effects of antibiotic molecules (even in antibiotic-resistant bacteria). If this is confirmed by future studies, these compounds may be useful for repurposing/reactivating current clinical antibiotics. Indeed, a recent study from our group reported that extracts prepared in a similar manner to those studied herein were effective in reactivating several antibiotics against ESBL and MRSA bacterial strains. The potentiating effects of the *T. ferdinandiana* extract components needs to be investigated in greater depth and future studies are planned in our group to explore this possibility.

This study did not identify the individual compounds that contribute to the antibacterial activity of *T. ferdinandiana* fruit. Several other studies have examined the phytochemistry of the fruit and have reported an abundance of several interesting classes of compounds, including tannins and flavonoids [as reviewed in][7]. Instead, our study compared general physio-chemical properties of the *T. ferdinandiana* fruits from both regions. Interestingly, the greater antibacterial potency of the NT fruit extracts correlated to substantially higher ascorbic acid levels in that fruit, indicating that the antibacterial mechanism may be associated with modulation of redox state. Previous studies have also reported similar redox-related effects for *T. ferdinandiana* extracts against the gastrointestinal protozoal parasite, *Giardia duodenalis* and linked the activity to the antioxidant capacity (and the extremely high ascorbic acid content in particular) of the extracts.[24] To determine whether similar effects contribute to the antibacterial potency of this extract, antibacterial compounds should be isolated, identified and then tested in combination with ascorbic acid to evaluate the class(es) of their interactions. All extracts were nontoxic ion the *Artemia* nauplii toxicity assay, indicating the safety for therapeutic usage. Whilst the *Artemia* nauplii toxicity assay is robust and generally correlates well with cellular toxicity assays, further testing against an extensive panel of mammalian cell lines is required to confirm the safety of these extracts for therapeutic use.

CONCLUSION

Methanolic extracts prepared using *T. ferdinandiana* fruit derived from both the NT and northern regions of Western Australia displayed substantial antibacterial activity, although the NT fruit was more potent. Interestingly, the antibacterial potency of the extracts correlated with their antioxidant capacities, and particularly with their ascorbic acid levels. It is unlikely that ascorbic acid alone is responsible for the antibacterial potency reported herein, as similar effects have not previously been reported for the pure compound. Instead, ascorbic acid may function as a potentiator, enhancing the activity of other compounds such as the tannins and flavonoids. Further studies are required to identify the active compounds and antibacterial mechanisms, as well as testing the potentiating effects of ascorbic acid on other *T. ferdinandiana* compounds. Additionally, whilst the *Artemia* nauplii toxicity assays used in our study indicate that the extracts are nontoxic, further studies using several mammalian cell lines are required to further confirm the safety of these extracts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Aq: aqueous; **CFU:** colony forming units; **CHL:** chloramphenicol; **CIP:** ciprofloxacin; **DMSO:** dimethyl sulfoxide; **ERY:** erythromycin; **LC_{50}:** dose causing 50% lethality; **MDR:** multi-drug resistant; **MeOH:** methanol; **MH:** Mueller Hinton; **MIC:** minimum inhibitory concentration; **MRSA:** methicillin-resistant *Staphylococcus aureus*; **NT:** Northern Territory; **ORAC:** oxygen radical absorbance capacity; **PEN:** penicillin; **SEM:** standard error of the mean; **TET:** tetracycline; **WA:** Western Australia(n); **ZOI:** zone of inhibition.

SUMMARY

- *Terminalia ferdinandiana* fruit extracts (aqueous and methanolic) from the Northern Territory (NT) and Western Australia (WA) regions of Australia were compared for their antibacterial and antioxidant properties
- The NT extracts were more potent against a panel of bacteria compared with the WA extracts
- The higher antibacterial activities of the NT extracts correlated with their higher antioxidant capacities
- All extracts were deemed nontoxic using *Artemia nauplii* assays

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