Evaluation of the antibacterial activity and toxicity of *Terminalia ferdinandia* fruit extracts

I. E. Cock[a,b]*, S. Mohanty[a]

[a]*Biomolecular and Physical Sciences, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Brisbane, Queensland 4111, Australia.*
[b]*Genomics Research Centre, Gold Coast Campus, Griffith University, Parklands Drive, Southport, Queensland 4222, Australia*

ABSTRACT

Introduction: *Terminalia ferdinandiana* is an endemic Australian native plant with a history of use as a food and as a medicinal agent by indigenous Australians. Yet the medicinal bioactivities of this plant are poorly studied. In the current study, solvent extracts from *T. ferdinandiana* fruit pulp were tested for antimicrobial activity and toxicity *in vitro*. **Results:** All extracts displayed antibacterial activity in the disc diffusion assay. The methanol extract proved to have the broadest specificity, inhibiting the growth of 13 of the 14 bacteria tested (92.9%). The deionised water extract inhibited the growth of 11 of the 14 bacteria tested (78.6%). The ethyl acetate, chloroform and hexane extracts inhibited 21.4%, 28.6% and 14.3% respectively. *T. ferdinandiana* methanolic extracts were approximately equally effective against Gram-positive (100%) and Gram-negative bacteria (90%). All other extracts were more effective at inhibiting the growth of Gram-positive bacteria. The water, ethyl acetate, chloroform and hexane extracts inhibited the growth of 100, 50, 50 and 50% Gram-positive bacteria respectively. In contrast, they inhibited the growth of 70, 10, 20 and 0% Gram-negative respectively. All *T. ferdinandiana* extracts were either non-toxic (ethyl acetate, chloroform, hexane) with no significant increase in mortality induction, or of low toxicity (LC_{50} >1000 µg/ml) (methanol, deionised water) in the *Artemia fransiscana* bioassay. **Conclusions:** The low toxicity of the *T. ferdinandiana* extracts and their inhibitory bioactivity against bacteria validate Australian Aboriginal usage of *T. ferdinandiana* and indicates its medicinal potential as well as its potential as a source of natural ascorbic acid.

Key words: *Terminalia ferdinandiana*, Kakadu plum, antibacterial, medicinal plants, phytotoxicity, superfoods

INTRODUCTION

Terminalia ferdinandiana (commonly known as Kakadu plum, gubinge, bush plum, billy goat plum and salty plum) is a moderately sized semi-deciduous tree of the family Combretaceae.[1] It is endemic to Australia, occurring predominantly in the tropical grasslands of the Northern Territory and the Kimberley region of Western Australia. *T. ferdinandiana* flowers at the end of dry season (September-November) and develops fruits from the middle of the wet season (January-June) to the early part of dry season. The fruit are 1.5 to 2 cm long ovoid shaped smooth fleshy drupes with a short beak at the tip. They become yellow to green in colour when ripe. The fruit have been used as a food source by Australian Aborigines in the northern regions of Australia for thousands of years.[2-4] They are astringent and have a pleasant but tart, slightly bitter flavour when eaten fresh[5] and are increasingly being used to produce powders, sauces, jams, beverages and preserves, as well as in cosmetic products.

T. ferdinandiana also has a history of use as a traditional medicine for the treatment of numerous ailments. The fruit were eaten by Australian Aborigines on long treks or hunting trips and was considered more valuable as a medicine rather than as a food.[6-8] The inner bark of the tree was also used medicinally to treat a variety of skin disorders and infections including wounds, sores and boils.[2] It is also effective in controlling fungal infections such as ringworm, and in the treatment of bacterial infections including its use in treating leprosy.[2]

Recently, *T. ferdinandiana* has been attracting attention due to its interesting phytochemistry. In particular, extremely high levels of ascorbic acid (vitamin C) have been reported for *T. ferdinandiana* fruit.[9,10] Indeed, *T. ferdinandiana* is now known as the richest source of vitamin C of any fruit in the world, with levels over 900 times higher than the same weight of blueberries.[10] Some studies have estimated the levels of

Address for correspondence:
Tel.: +61 7 37357637; fax: +61 7 37355282.
E-mail: I.Cock@griffith.edu.au (I. E. Cock).

DOI: **

ascorbic acid in *T. ferdinandiana* fruit to be as high as 5.5% of dry weight, in comparison to approximately 0.5% dry weight in oranges, grapefruit and limes.[3] Ascorbic acid is well known for its ability to scavenge free radicals and thereby reduce oxidative stress.[11] As the induction of oxidative stress is known to be associated with some cancers, cardiovascular disease, neurodegeneration disorders, diabetes and obesity,[12] the high levels of ascorbic acid associated with *T. ferdinandiana* fruit may also have beneficial health related bioactivities.

Phytochemical studies of the nutritional value of *T. ferdinandia* fruit have also shown it to also be high in other important polyphenolic antioxidants including ellagic and gallic acids.[13] Pure ellagic and gallic acids and their derivatives have previously been shown to have antibacterial,[14,15] antifungal,[16,17] antiviral,[18] anti-inflammatory,[19] antimutagenic,[20] and antiallergic bioactivities.[21] Furthermore, ellagic and gallic acids have demonstrated cytotoxic activity towards cancer cells, whilst being nontoxic to normal cell lines.[22-23]

Given the previous phytochemical studies, it is surprising that the therapeutic potential of *T. ferdinandiana* remains largely unstudied. Most of the studies regarding this plant solely report on the vitamin C level and the total antioxidant capacity without examining medicinally important bioactivities. Therefore, the current study reports on the antibacterial properties of *T. ferdinandiana* fruit pulp extracts as well as examining their toxicity to determine their potential as antibiotic agents and to validate the ethnopharmacological usage by Australian Aborigines from northern regions of Australia.

MATERIALS AND METHODS

Plant material

T. ferdinandiana *fruit pulp samples*

T. ferdinandiana fruit pulp was a gift from David Boehme of Wild Harvest, Northern Territory, Australia. The pulp was frozen for transport and stored at –10°C until processed.

Preparation of crude extracts

T. ferdinandiana fruit pulp was thawed at room temperature and dried in a Sunbeam food dehydrator. The dried pulp material was subsequently ground to a coarse powder. 1 g of each of the ground dried pulp was extracted extensively in 50 ml of either methanol, deionised water, ethyl acetate, chloroform or hexane for 24 hours at 4°C with gentle shaking. All solvents were supplied by Ajax and were AR grade. The extracts were filtered through filter paper (Whatman No. 54) under vacuum followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant pellets were dissolved in 10 ml deionised water. The extract was passed through 0.22 µm filter (Sarstedt) and stored at 4°C.

Antibacterial screening

Test microorganisms

All microbial strains were obtained from Michelle Mendell and Tarita Morais, Griffith University, Australia. Stock cultures of *Aeromonas hydrophilia*, *Alcaligenes feacalis*, *Bacillus cereus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas fluorescens*, *Salmonella newport*, *Serratia marcescens*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were subcultured and maintained in nutrient broth at 4°C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified Kirby-Bauer disc diffusion method.[24] Briefly, 100 µl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml. 100 µl of microbial suspension was spread onto nutrient agar plates.

The extracts were tested using 5 mm sterilised filter paper discs. Discs were impregnated with 10 µl of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4°C for 2 hours before incubation with the test microbial agents. Plates inoculated with *Alcaligenes feacalis*, *Aeromonas hydrophilia*, *Bacillus cereus*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Proteus mirabilis, Pseudomonas fluorescens* and *Serratia marcescens* were incubated at 30°C for 24 hours, then the diameters of the inhibition zones were measured in millimetres. Plates inoculated with *Escherichia coli*, *Salmonella newport*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were incubated at 37°C for 24 hours, then the diameters of the inhibition zones were measured. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in at least triplicate. Mean values are reported in this study. Standard discs of ampicillin (2 µg) and chloramphenicol (10 µg) were obtained from Oxoid Ltd. and served as positive controls for antimicrobial activity. Filter discs impregnated with 10 µl of distilled water were used as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of the *T. ferdinandiana* extracts was determined by the disc diffusion method across a range of doses. The plant extracts were diluted in deionised water across a concentration range of 5 mg/ml to 0.1 mg/ml. Discs were impregnated with 10 µl of the test 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 for each extract. Linear regression was used to calculate the MIC values.

Toxicity Screening

Reference toxins for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/ml solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay. Mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) was obtained from Sigma-Aldrich as a mixture of cis (76.6%) and trans (23.0%) isomers and prepared as a 4 mg/ml stock in distilled water. The stock was serially diluted in artificial seawater for use in the bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using the *Artemia franciscana* nauplii lethality assay developed by Meyer et al[25] for the screening of active plant constituents with the following modifications. *Artemia franciscana* Kellogg cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Synthetic seawater was prepared using Reef Salt, AZOO Co., USA. Seawater solutions at 34 g/l distilled water were prepared prior to use. 2 g of *A. franciscana* cysts were incubated in 1 l synthetic seawater under artificial light at 25°C, 2000 Lux with continuous aeration. Hatching commenced within 16-18 h of incubation. Newly hatched *A. franciscana* (nauplii) were used within 10 h of hatching. Nauplii were separated from the shells and remaining cysts and were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel and the nauplii rich water closest to the light was removed for biological assays. 400 µl of seawater containing approximately 42 (mean 41.6, $n = 150$, SD 17.8) nauplii were added to wells of a 48 well plate and immediately used for bioassay. The plant extracts were diluted to 2 mg/ml in seawater for toxicity testing, resulting in a 1 mg/ml concentration in the bioassay. 400 µl of diluted plant extracts and the reference toxins were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A negative control (400 µl seawater) was run in at least triplicate for each plate. All treatments were performed in at least triplicate. 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 observed within 10 seconds. After 72 h all nauplii were sacrificed and counted to determine the total number per well. The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.[26]

RESULTS

Antibacterial Activity

1 kg of *T. ferdinandiana* fruit pulp was dehydrated resulting in 165 g of dried material. Extraction of 1 g of dried plant material with various solvents yielded dried plant extracts ranging from 23 mg to 498 mg (Table 1). Methanol and deionised water both gave high yields of dried extracted material (371 and 498 mg respectively) whilst ethyl acetate, chloroform and hexane all extracted relatively low masses (28, 60 and 23 mg respectively). The dried extracts were resuspended in 10 ml of deionised water resulting in the extract concentrations shown in Table 1.

10 µl of each extract (50 µg) was tested in the disc diffusion assay against 14 bacteria (Table 2). *T. ferdinandiana* fruit methanol extract was particularly effective as an antibacterial agent, inhibiting the growth of 13 of the 14 bacteria tested (92.9%). The deionised water extract also displayed broad antibacterial activity, inhibiting the growth of 11 of the 14 bacteria tested (78.6%). Ethyl acetate, chloroform and hexane extracts each had narrower specificity, inhibiting 3 (21.4%), 4 (28.6%) and 2 (14.3%) of the tested bacteria respectively.

Both Gram-positive and Gram-negative bacteria were affected approximately equally by the *T. ferdinandiana* pulp methanol extract (90% and 100% respectively). In contrast, all other extracts proved more effective at inhibiting the growth of Gram-positive bacteria. Of the 10 Gram-negative bacteria tested, 7 (70%) were inhibited by the *T. ferdinandiana* pulp deionised water extract whilst 100% of the Gram-positive bacterial growth was inhibited by this extract. The antibacterial specificity towards Gram-positive bacteria was even more evident for the ethyl acetate, chloroform and hexane extracts. The ethyl acetate extract inhibited the growth of 2 of the 4 Gram-positive bacteria tested (50%) and 1 of the 10 Gram-negative bacteria tested (10%). The chloroform and hexane extracts also inhibited the growth of 2 of the 4 Gram-positive bacteria tested (50%) each. In contrast, the chloroform extract inhibited the growth of 2 of the 10 Gram-negative bacteria tested (20%), whilst no Gram-negative bacterial growth was inhibited by the hexane extract (0%).

The relative level of antibacterial activity was evaluated by determining the MIC values for each extract against the bacteria which were shown to be susceptible by disc

Table 1: The mass of dried material extracted with the various solvents and the concentration after resuspension in deionised water

Solvent	Mass of Dried Extract (mg)	Resuspended Extract Concentration (mg/ml)
Methanol	371	37.1
Deionised Water	498	49.8
Ethyl Acetate	28	2.8
Chloroform	60	6
Hexane	23	2.3

diffusion assays. MIC's were evaluated in the current studies by disc diffusion across a range of concentrations. This has previously been determined to be a valid method of MIC determination as MIC values determined by disc diffusion correlate well with those determined by broth dilution assays.[27]

The methanol extract was particularly effective at inhibiting the growth of *A. faecalis*, *P. mirabilis*, *P. fluorescens*, *S. newport* and *S. pyogenes*, as seen by minimum inhibitory concentration (Table 3). Indeed, the growth of these bacteria was inhibited by low concentrations (<100 µg/ml) of the extract. *A. hydrophilia*, *C. freundi*, *S. sonnei*, *B. cereus*, *S. aureus* and *S. epidermidis* also were quite susceptible, displaying inhibited growth at concentrations below 500 µg/ml. *P. mirabilis* growth was also inhibited by low concentrations (<100 µg/ml) of the deionised water extract, whilst *C. freundi*, *E. coli*, *P. fluorescens* and *S. epidermidis* were all inhibited by the water extract at concentrations below 500 µg/ml.

Qantification of toxicity

The *T. ferdinandiana* fruit extracts (Figures 1a-e) were diluted in artificial seawater for toxicity testing in the *Artemia franciscana* lethality bioassay. For comparison, the reference

Table 2: Antibacterial activity of *T. ferdinandiana* fruit extracts measured as zones of inhibition (mm)

	Methanol extract	Water extract	Ethyl acetate extract	Chloroform extract	Hexane extract	Ampicillin	Chloramphenicol	Negative control (water)
Gram negative rods								
A. faecalis	13.0 ± 0	–	–	–	–	15.2 ± 1.2	6.3 ± 0.6	–
A. hydrophilia	8.0 ± 0	7.3 ± 1.2	–	6.0 ± 0	–	12.0 ± 1.0	28.7 ± 1.6	–
C. freundi	12.7 ± 1.2	13.6 ± 1.2	–	–	–	8.3 ± 0.6	15.7 ± 1.2	–
E. coli	8.3 ± 0.6	7.8 ± 1.0	–	–	–	14.7 ± 0.6	17.3 ± 0.6	–
K.pneumoniae	6.0 ± 0	–	–	–	–	10.3 ± 0.6	21.3 ± 1.5	–
P. mirabilis	14.7 ± 1.5	12.3 ± 0.6	7.0 ± 1.0	7.7 ± 0.6	–	17.3 ± 0.6	8.7 ± 0.6	–
P. fluorescens	12.7 ± 0.6	9.7 ± 0.6	–	–	–	18.2 ± 0.5	21.2 ± 1.2	–
S. newport	12.7 ± 0.6	7.0 ± 0	–	–	–	18.7 ± 0.6	20.3 ± 0.6	–
S. marcescens	–	–	–	–	–	0 ± 0	14.7 ± 0.6	–
S. sonnei	9.7 ± 0.6	7.3 ± 0.6	–	–	–	14.0 ± 0	14.3 ± 0.6	–
Gram positive rods								
B. cereus	11.7 ± 0.6	7.3 ± 0.6	–	–	–	26.7 ± 0.6	13.3 ± 1.2	–
Gram positive cocci								
S. aureus	8.3 ± 0.6	6.6 ± 0.6	6.0 ± 0	7.2 ± 1.0	6.7 ± 0.6	11.7 ± 2.1	16.0 ± 1.0	–
S. epidermidis	10.7 ± 0.6	14.3 ± 0.6	9.0 ± 0	6.0 ± 0	6.3 ± 0.6	26.3 ± 1.5	12.3 ± 0.6	–
S. pyogenes	12.7 ± 1.2	7.3 ± 0.6	–	–	–	17.0 ± 1.0	24.0 ± 1.0	–

Numbers indicate the mean diameters (mm) of inhibition of triplicate experiments ± standard deviation. – indicates no growth inhibition. Chloramphenicol (10 µg) and ampicillin (2 µg) were used as the positive controls.

Table 3: Minimum inhibitory concentrations (µg/ml) of *T. ferdinandiana* extracts against susceptible bacteria

	Methanol	Water	Ethyl Acetate	Chloroform	Hexane
A. faecalis	46.8	–	–	–	–
A. hydrophilia	160.2	518.8	–	695.5	–
C. freundi	159.4	287.1	–	–	–
E. coli	684.3	348.8	–	–	–
K. pneumoniae	924.7	–	–	–	–
P. mirabilis	29.1	85.9	500	672.3	–
P. fluorescens	47.3	147.1	–	–	–
S. newport	35	875.7	–	–	–
S. sonnei	112.5	566.6	–	–	–
B. cereus	113.5	530.9	–	–	–
S. aureus	285.6	756.8	825.7	707.1	594.6
S. epidermidis	114.7	196.7	739.7	695.6	347.9
S. pyogenes	47.6	250	–	–	–

Numbers indicate the mean MIC values of at least triplicate determinations. – indicates no growth inhibition.

toxins potassium dichromate (800 µg/ml) (Figure 1g) and Mevinphos (2000 µg/ml) (Figure 1h) were also tested in the *Artemia franciscana* lethality bioassay. The potassium dichromate and Mevinphos reference toxins were much more rapid in their onset of mortality than any of the *T. ferdinandiana* extracts at the concentrations tested. For both reference toxins, the induction of mortality was seen within the first 3 hours of exposure. 100% mortality was

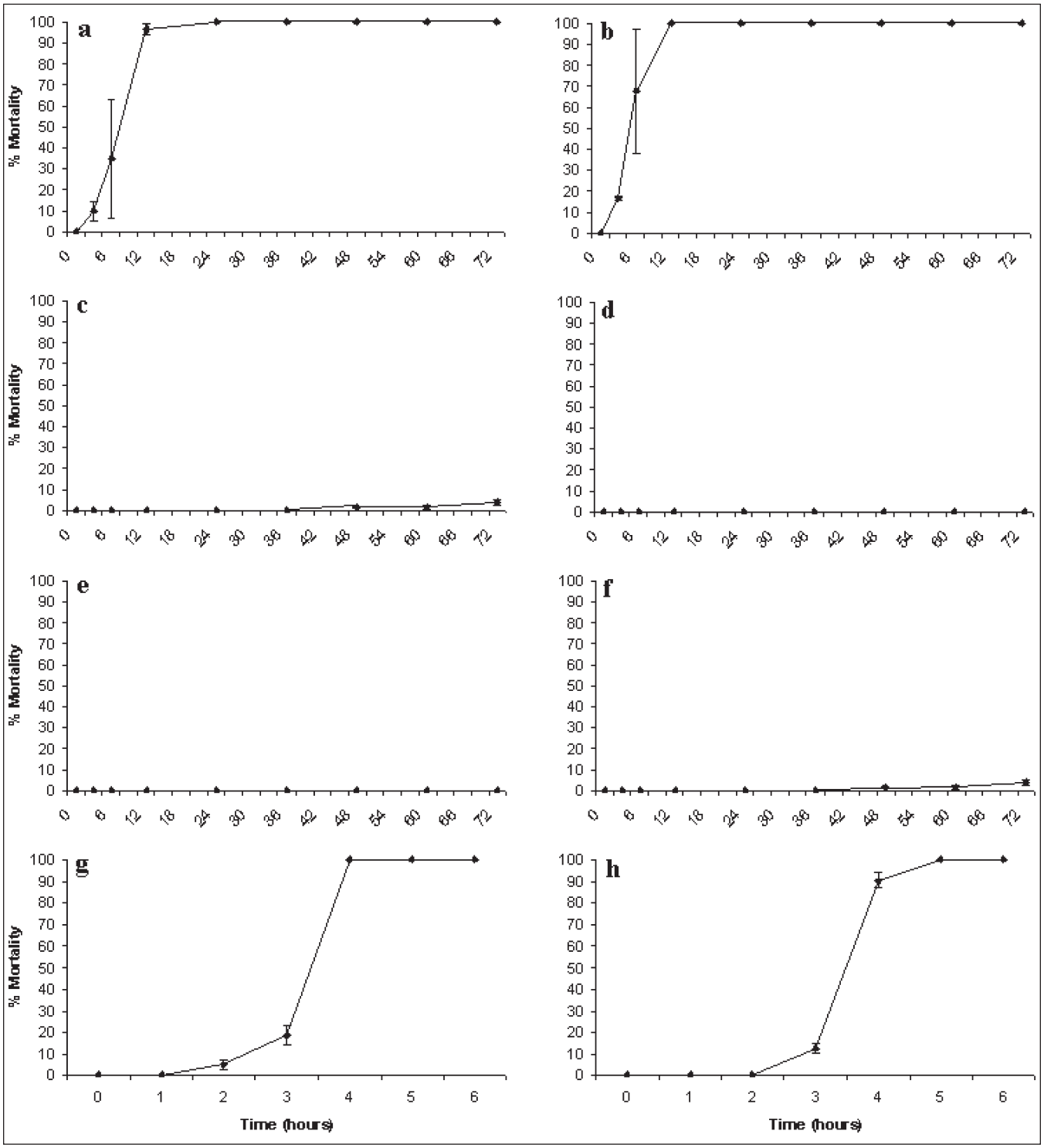

Figure 1: Brine shrimp lethality of (a) *T. ferdinandiana* fruit methanol extract (1000 µg/ml), (b) *T. ferdinandiana* fruit water extract (1000 µg/ml), (c) *T. ferdinandiana* fruit ethyl acetate extract (1000 µg/ml), (d) *T. ferdinandiana* fruit chloroform extract (1000 µg/ml), (e) *T. ferdinandiana* fruit hexane extract (1000 µg/ml), (f) artificial seawater negative control, (g) potassium dichromate (800 µg/ml), (h) Mevinphos (2000 µg/ml). All bioassays were performed in at least triplicate and are expressed as mean ± standard deviation.

Table 4: LC_{50} (95% confidence interval) for *A. franciscana* nauplii exposed to *T. ferdinandiana* leaf and fruit extracts, the reference toxins potassium dichromate and Mevinphos and a seawater control

	LC50				
	3 hours	6 hours	24 hours	48 hours	72 hours
Methanol	1964	1473	913	900	886
Water	1963	1050	900	900	900
Ethyl Acetate	–	–	–	–	–
Chloroform	–	–	–	–	–
Hexane	–	–	–	–	–
Potassium Dichromate	–	286	92	86	83
Mevinphos	–	1286	1004	525	109
Seawater Control	–	–	–	–	–

– denotes values that were not obtained as ≥ 50% mortality was not obtained at this time point.

evident following 4 hours of exposure. In contrast, mortality due to *T. ferdinandiana* methanol and water extract exposure was evident within 6 hours and 12 hours was required to achieve approximately 100% mortality. None of the other extracts induced mortality above the levels seen for seawater controls at any time tested.

To determine the effect of toxin concentration on the induction of mortality, the LC_{50} values of the extracts was determined by testing across the concentration range 2000 µg/ml to 15 µg/ml in the *Artemia* nauplii bioassay. For comparison, potassium dichromate and Mevinphos were tested across the same concentration range. Table 4 shows the LC_{50} values of *T. ferdinandiana* extracts towards *A. franciscana*. No LC_{50} values are reported for the *T. ferdinandiana* ethyl acetate, chloroform or hexane fruit extracts as no increase in mortality above the seawater controls was seen for these extracts at any time tested. The *T. ferdinandiana* methanol and water extracts displayed similar toxicity to Mevinphos at 24 hours but were substantially less toxic at 48 and 72 hours with 48 hour LC_{50} values of 900 µg/ml for both the methanol and water extracts and 72 hour LC_{50} values of 886 µg/ml and 900 µg/ml respectively, compared to 48 h and 72 h LC_{50} values of 525 µg/ml and 109 µg/ml for Mevinphos. Potassium dichromate was substantially more toxic at 24 hours (24 h LC_{50} 92 µg/ml), 48 hours (48 h LC_{50} 86 µg/ml) and 72 hours (72 h LC_{50} 83 µg/ml).

DISCUSSION

The current study reports on the antimicrobial activity and toxicity of *T. ferdinandiana* fruit pulp extracts. The ability of *T. ferdinandiana* fruit pulp extracts to inhibit the growth of both Gram-positive and Gram-negative bacteria seen in this study is in agreement with previous reports of the antibacterial activity of other plants used by Australian Aborigines as antibacterial agents. The antiseptic properties of *Eucalypts*,[28-31] *Leptospermums*,[32-35] and *Melaleucas*,[36,37] have been extensively studied and shown to inhibit the growth of a wide variety of both Gram-positive and Gram-negative bacteria.

We report *T. ferdinandiana* fruit pulp solvent extracts to have greater antibacterial activity towards Gram-positive bacteria than towards Gram-negative bacteria in this study. The greater susceptibility of Gram-positive bacteria towards the *T. ferdinandiana* fruit extracts is in agreement with previously reported results for South American,[38] African,[39-40] and Australian[41] plant extracts. Results within this laboratory have also confirmed the greater susceptibility of Gram-positive bacteria towards other Australian plant extracts.[28] The Gram-negative bacterial cell wall outer membrane is thought to act as a barrier to many substances including antibiotics.[42] The uptake of the *T. ferdinandiana* extract antibiotic agents by Gram-negative bacteria is presumably affected by the cell wall outer membrane of some bacteria.

Individual *T. ferdinandiana* fruit pulp components responsible for the extracts antiseptic potential were not identified in the current study. However, previous reports have demonstrated that *T. ferdinandiana* contain high levels of antioxidants.[9,10,13] In particular, several studies have highlighted the fact the *T. ferdinandiana* has the highest recorded concentrations of ascorbic acid of any fruit in the world.[9,10] However, it is unlikely that ascorbic acid alone is responsible for the broad antibacterial activity and low MIC values seen during this study, even at the high levels present in *T. ferdinandiana* fruit. Previous studies have demonstrated that ascorbic acid alone displays only weak antibacterial activity towards *E. coli* and *S. aureus*, even at relatively high concentrations.[43] Instead, if ascorbic acid is involved in the antibacterial bioactivities reported here, it is more likely that it works in a synergistic manner with other *T. ferdinandiana* extract phytochemicals. Ascorbic acid has previously been shown to enhance the antibacterial activity of other polyphenolic compounds through an inhibition of the oxidation of these polyphenols. For

example, epigallocatechin gallate (EGCG), the most abundant polyphenol of tea leaves (*Camellia sinensis*) has well established inhibitory activity towards *S. aureus* growth although this activity is unstable due to oxidation.[44,45] The addition of ascorbic acid to EGCG solutions has been shown to significantly enhance their antibacterial activity and to prolong their inhibitory effect.[46]

Gallic and ellagic acids, as well as their derivatives, have been reported to be present in *T. ferdinandiana* fruit.[13] As these compounds have well established antibacterial activities,[14,15] they may be responsible, at least in part, for the bacterial growth inhibitory effects of *T. ferdinandiana* fruit reported here. Similarly, gallic and ellagic acids also have well documented antifungal,[16,17] antiviral,[18] anti-inflammatory,[19] antimutagenic,[20] antiallergic[21] and anticancer[22,23] bioactivities. Further studies to examine *T. ferdinandiana* fruit extracts against these bioactivities is also warranted.

T. ferdinandiana fruit has also been reported to contain a number of other important phytochemical components, vitamins and nutrients which could contribute to medicinally important bioactivities of this plant, including antibacterial activity. Whilst *T. ferdinandiana* fruit extracts are not yet fully characterised due to difficulties in separating some components, high levels of antioxidant molecules have been reported. Apart from the high ascorbic, gallic and ellagic acid levels previously discussed, *T. ferdinandiana* fruit also contains high levels of phenolic compounds. Indeed, phenolic compound levels nearly 5 fold higher than in blueberries have previously been demonstrated to be associated with polar *T. ferdinandiana* fruit extracts.[9] These authors noted *T. ferdinandiana* fruit to be very rich in chlorophyll a and also to have high levels of chlorophyll b. Both chlorophyll a and b have previously been shown to be capable of relieving oxidative stress.[47] Lipophilic *T. ferdinandiana* fruit extracts are rich in lutein (a carotenoid antioxidant compound associated with eye health) and with vitamin E and vitamin E analogues.[9] Other antioxidants present in *T. ferdinandiana* fruit include the glucosides quercetin and hesperitin, and the glycosides kaempferol and luteolin.[9] *T. ferdinandiana* fruit is also a good source of the minerals magnesium, zinc, calcium, potassium, sodium, iron, phosphorous, manganese, copper and molybdenum.[9] Of further interest, the same study also noted a high potassium/sodium ratio in *T. ferdinandiana* fruit.[9] As high potassium/sodium ratios have been shown to relieve hypertension,[48] testing the effect of *T. ferdinandiana* fruit on individuals suffering from this condition is also warranted.

The findings reported here also indicate that *T. ferdinandiana* fruit extracts display low toxicity towards *Artemia franciscana*. Indeed, the ethyl acetate, chloroform and hexane extracts did not induce mortality above that seen for the seawater control at any dose or time tested. Only the methanol and deionised water extracts were seen to induce mortality above that of the seawater controls and even this is considered low toxicity. Both of these extracts displayed 24, 48 and 72 h LC_{50} values of approximately 900 µg/ml. As an LC_{50} of ≥1000 µg/ml is defined as nontoxic,[25] these extracts are considered of only low toxicity. Toxicity towards *A. franciscana* has also previously been shown to correlate well with toxicity towards human cells for some toxins.[49] Therefore, studies into potential anticancer activities of *T. ferdinandiana* fruit extracts are warranted, particularly for the methanol and water extracts.

In conclusion, this study focussed on the bacterial growth inhibitory potential of *T. ferdinandiana* fruit pulp. Other studies are needed to examine other medicinally important bioactivities of *T. ferdinandiana* fruit. The results of the current study indicate that *T. ferdinandiana* fruit pulp extracts are worthy of further study due to their antibacterial activity. Evaluation of *T. ferdinandiana* fruit pulp extract antibacterial properties against a more extensive panel of microbial agents is warranted. Likewise, purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents. Whilst the extracts examined in this report are promising as antimicrobial agents, caution is needed before these compounds can be applied to medicinal purposes and as food additives to inhibit spoilage. In particular, further toxicity studies using human cell lines are needed to determine the suitability of these extracts for these purposes.

ACKNOWLEDGEMENTS

Financial support for this work was provided by the School of Biomolecular and Physical Sciences, Griffith University, Australia. The authors would like to thank David Boehme of Wild Harvest, Northern Territory, Australia for providing the *T. ferdinandiana* fruit pulp used in these studies.

REFERENCES

1. Brock J. Top End Native plants: A Comprehensive Guide to the Trees and Shrubs of the Top End of the Northern Territory. 2001; Reed books, Sydney, Australia.
2. Gorman JT, Griffiths AD, Whitehead PJ. An analysis of the use of plant products for commerce in remote Aboriginal communities of Northern Australia. Econ Bot 2006; 60(4):362-373.
3. Woods B. A study of the intra-specific variantions and commercial potential of *Terminalia ferdinandiana (Excell)* (the Kakadu Plum). 1995; M.Sc. Thesis. Northern Territory University, Darwin.
4. Brand JC, Rae C, McDonnell J, Lee A, Cherikoff V, Truswell AS. The nutritional composition of Australian Aboriginal bushfoods. Food Technology in Australia 1982; 35:293-298.
5. Smythe H. Defining the unique flavours of Australian native foods. Rural Industry Research and Development 2010; publication number 10/062, ACT, Australia.

6. Clarke PA. Aboriginal people and their plants. 2007; Rosenberg publishing Pty Ltd, Kenthurst, NSW, Australia.
7. Isaacs J. Bush Food. 1987; Weldons Pty Ltd, Australia.
8. Hegarty MP, Hegarty EE. Food Safety of Australian Plant Bushfoods, Rural Industries Research and Development Corporation 2001; publication number 01/28, ACT, Australia.
9. Konczak I, Zabaras D, Dunstan M, Aguas P. Antioxidant capacity and hydrophilic phytochemicals in commercially grown native Australian fruits, Food Chem 2010; 123:1048-1054.
10. Netzel M, Netzel G, Tian Q, Schwartz S, Konczak I. Native Australian fruits – a novel source of antioxidants for food, Innov Food Sci Emerg Technol 2007; 8:339-346.
11. Guaiquil VH, Vera HC, Golde DW. Mechanism of Vitamin C Inhibition of Cell Death Induced by Oxidative Stress in Glutathione-depleted HL-60 Cells. J Biol Chem 2001; 276 (44):40955-40961.
12. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219:1-14.
13. Cherikoff V, Kowalski G. Superfoods for Superhealth. Discover the Wonders of Australian Native Fruits. 2008; Excerp available online at http://www.kakadujuice.com/clientinc/upload/Promotional.Tools/SuperFoods%20Excerpt.pdf. Accessed 7/10/ 2010.
14. Hancock V, Dahl M, Munk Vejborg R, Klemm P. Dietary plant components ellagic acid and tannic acid inhibit *Escherichia coli* biofilm formation. J of Med Microbiol 2010; 59:496-498.
15. Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA. Antibacterial activity directed isolation of compounds from *Punica granatum,* J Food Sci 2007; 72 (9):341-345.
16. Ahn YJ, Lee HS, Oh HS, Kim HT, Lee YH. Antifungal activity and mode of action of Galla rhois-derived phenolics against phytopathogenic fungi. Pestic Biochem Physiol 2005; 81:105-112.
17. Kubo I, Xiao P, Fujita K. Antifungal activity of octyl gallate: Structural criteria and mode of action. Bioorg Med Chem Lett 2001; 11:347-350.
18. Kang EH, Kown TY, Oh GT, Park WF, Park SI, Park SK, Lee YI. The flavonoid ellagic acid from a medicinal herb inhibits host immune tolerance induced by the hepatitis B virus-e antigen. Antiviral Res 2006; 72(2):100-106.
19. Papoutsi Z, Kassi E, Chinou I, Halabalaki M, Skaltsounis LA, Moutsatsou P. Walnut extract (*Juglans regia* L.) and its component ellagic acid exhibit anti-inflammatory activity in human aorta endothelial cells and osteoblastic activity in the cell line KS483. Br J Nutr 2008; 99:715-722.
20. Narayanan BA, RE GG. IGF-II down regulation associated cell cycle arrest in colon cancer cells exposed to phenolic antioxidant ellagic acid. Anticancer Res 2001; 21 (1A):359-364.
21. Choi YH, Yan GH. Ellagic acid attenuates immunoglobulin E-mediated allergic response in mast cells. Biol Pharm Bull 2009; 32:1118-1121.
22. Losso JN, Bansode RR, Trappey A, Bawadi HA, Truax R. In vitro anti-proliferative activities of ellagic acid. J Nutr Biochem 2004; 15:672-678.
23. Ohno Y, Fukuda K, Takemura G, Toyota M, Watanabe M, Yasuda N, Xinbin Q, Maruyama R, Akao S, Gotou K, Fujiwara T, Fujiwara H. Induction of apoptosis by gallic acid in lung cancer cells. Anticancer Drugs 1999; 10:845-851.
24. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Path 1966; 45:493-496.
25. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 1982; 45:31-34.
26. Finney DJ. Probit Analysis. 1971 3rd ed., Cambridge University Press, Cambridge.
27. Gaudreau C, Girouard Y, Ringuette L, Tsimiklis C. Comparison of disc diffusion and agar dilution methods for erythromycin and ciprofloxacin susceptibility testing of *Campylobacter jejuni* subsp. *Jejuni*, Antimicrob Agents Chemother 2007; 1524-1526.
28. Cock IE. Antimicrobial activity of *Eucalyptus major* and *Eucalyptus baileyana* methanolic extracts. Internet J Microbiol 2008a; 6, 1.
29. Sartorelli P, Marquioreto AD, Amaral-Baroli A, Lima MEL, Moreno PRH. Chemical composition and antimicrobial activity of the essential oils from two species of *Eucalyptus*. Phytother Res 2007; 21:231-233.
30. Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol 2002; 74:101-109.
31. Oyedeji AO, Ekundayo O, Olawore ON, Adeniyi BA, Koenig WA. Antimicrobial activity of the essential oils of five *Eucalyptus* species growing in Nigeria. Fitoterapia 1999; 70:526-528.
32. Cock IE. Antibacterial activity of selected Australian native plant extracts. The Internet J Microbiol 2008b; 4, 2.
33. Davis C, Ward W. Control of Chalkbrood disease with natural products, Rural Industries Research and Development Corporation 2003; Canberra, Australia.
34. Weston RJ, Brocklebank LK, Lu Y. Identification of quantitative levels of antibacterial components of some New Zealand honeys. Food Chem 2000; 70:427-435.
35. Setzer MC, Setzer WN, Jackes BR, Gentry GA, Moriarity DM. The medicinal value of tropical rainforest plants from Paluma, North Queensland, Australia. Pharm Biol 2001; 39:1, 67-78.
36. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. Clin Microbiol Rev 2006; 19:1, 50-62.
37. Papadopoulos CJ, Carson CF, Hammer KA, Riley TV. Susceptibility of pseudomonads to *Melaleuca alternifolia* (tea tree) oil and components. J Antimicrob Chemother 2006; 58:449-451.
38. Paz EA, Cerdeiras MP, Fernandez J, Ferreira F, Moyna P, Soubes M, Vazquez A, Vero S, Zunino L. Screening of Uruguayan medicinal plants for antimicrobial activity. J Ethnopharmacol 1995; 45:67-70.
39. Kudi AC, Umoh JU, Eduvie LO, Gefu J. Screening of some Nigerian medicinal plants for antibacterial activity. J Ethnopharmacol 1999; 67:225-228.
40. Vlietinck AJ, van Hoof L, Totte J, Lasure A, Vanden Berghe D, Rwangabo PC, Mvukiyumwani J. Screening of a hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J Ethnopharmacol 1995; 46:31-47.
41. Palombo EA, Semple SJ. Antibacterial activity of traditional Australian medicinal plants. J Ethnopharmacol 2001; 77:151-157.
42. Tortora GJ, Funke BR, Case CL. Microbiology: An Introduction 2001; Benjamin Cummings, San Francisco.
43. Hismiogullari SE, Hismiogullari AA, Sahin F, Toksoy Oner E, Yenice D, Karasartova D. Investigation of the antibacterial and cytotoxic effects of organic acids including ascorbic acid, lactic acid and acetic acids on mammalian cells. J Anim Vet Adv 2008; 6 (7):681-684.
44. Zhao WH, Hu ZQ, Hara Y, Shimamura T. Mechanism of synergy between epigallocatechin gallate and beta-lactams against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2002; 45:1737-1742.
45. Hamilton-Miller JM, Shah S. Activity of the tea component epicatechin gallate and analogues against methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2000; 46:852-853.
46. Hatano T, Tsugawa M, Kusuda M, Taniguchi S, Yoshida T, Shiota S, Tsuchiya T. Enhancement of antibacterial effects of eppigallocatechin gallate, using ascorbic acid. Phytochemistry 2008; 69:3111-3116.
47. Motilva MJ. Chlorophylls – from functionality in food to health relevance. In 5th Pigments in Food Congress - for quality & health, Proceedings of the 5th International Congress on Pigments in Food 2008; Helsinki, Finland, edited by Heinonen M, 69-73.
48. Adrogué HJ, Madias NE. Sodium and potassium in the pathogenesis of hypertension. New Engl J Med 2007; 356:1966-1978.
49. McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. Drug Inf J 1998; 32:513-524.