Australian Government
Rural Industries Research and Development Corporation

Functional Properties of Australian Bushfoods

A report for the Rural Industries Research and Development Corporation

by Jian Zhao and Samson Agboola

January 2007

RIRDC Publication No 07/030
RIRDC Project No UCS-29A

ISBN 1 74151 429 0
ISSN 1440-6845

Functional Properties of Australian Bushfoods
Publication No. 07/030
Project No. UCS-29A

Researcher Contact Details
Jian Zhao
School of Wine and Food sciences
Charles Sturt University
Locked Bag 588
Wagga Wagga NSW 2678

Phone: 02 69332968
Fax: 0269332107
Email: jzhao@csu.edu.au

IIn submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details
Rural Industries Research and Development Corporation
Level 2, 15 National Circuit
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4819
Fax: 02 6272 5877
Email: rirdc@rirdc.gov.au.
Web: http://www.rirdc.gov.au

Published in January 2007
Printed on environmentally friendly paper by Canprint

Foreword

The Australian bushfood industry has come a long way since its beginning in the early 1980s. The main force driving the growth of the industry over the past two decades has been the appeal of its nativeness and a distinctively Australian flavour. While this image should be carefully preserved, a sustainable growth strategy would require the industry to make significant inroads into the mainstream food markets by exploring alternative uses and markets beyond their traditional area of application.

Anecdotal evidence shows that many Australian bushfoods possess functional properties such as antimicrobial, antioxidant and emulsifying properties. These properties have broad areas of application with significant commercial values. This presents a unique opportunity for value-adding and market expansion for the industry. However, knowledge in this area is rudimentary and obtained mainly through experience rather than through systematic, scientific investigation. This lack of systematic knowledge is being recognised as one of the main constraints of the industry.

This project seeks to close this knowledge gap by systematically examining the antimicrobial, antioxidant and emulsifying properties of a number of bushfoods, emphasising those with the greatest economic potential. The overall aim of the project is to enhance our understanding of the functional properties of bushfoods.

The research has generated a wealth of original data and made a number of significant findings that are relevant to the bushfood industry as well as the scientific and general communities.

This project was funded from RIRDC Core Funds which are provided by the Australian Government.

This report, an addition to RIRDC's diverse range of over 1500 research publications, forms part of our New Plant Products R&D program, which aims to facilitate the development of new industries based on plant or plant products that have commercial potential for Australia.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/fullreports/index.html
- purchases at www.rirdc.gov.au/eshop

Peter O'Brien
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

The authors wish to thank Mr Vic Cherikoff, Mr Gil Freeman, Ms Sibylla Hess-Buschmann, Mr Barry Lillywhite and Mr Peter Yates for providing bushfood samples and for their collaboration and support throughout this project.

We thank the following research assistants and students for their contribution to the experimental work and drafting of parts of this report: Milena Radovanovic-Tesic, Joel Thegerson, Ee Kah Yaw, Janice Foo, Kee Kee Goh, Beh Poe Hoe, Way Yi Tah and Silke Kühlken. We are grateful to the EH Graham Centre for Agricultural Innovation (formerly Farrer Centre for Agricultural Research) for providing scholarships to several of the students involved in the project.

We thank Dr Ata Rehman and Dr Philip Kerr for their expert advice on protein analysis and phytochemistry respectively.

Finally, the financial support provided by the Rural Industries Research and Development Corporation is gratefully acknowledged.

Abbreviations

ABTS	2,2'-azinobis (3-ethylbenzothialozinesulfonic acid)
AO	Antioxidant
CE	Capillary electrophoresis
Da	Dalton
DPPH	1,1-diphenyl-2-picrylhydrazyl
GAE	Gallic acid equivalent
GL	Gippsland
HPLC	High performance liquid chromatography
MRS	De Man, Rogosa and Sharpe
NR	Northern Rivers
Pa	Pascal
QEAC	Quercetin equivalent antioxidant capacity
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TEAC	Trolox equivalent antioxidant capacity
TROLOX	6–Hydroxy–2,5,7,8–tetramethylchroman–2–carboxylic Acid
UV/Vis	Ultraviolet/Visible

Contents

List of Tables

List of Figures

Executive Summary

What the report is about?
This report examines the functional properties of Australian bushfoods and provides detailed information on the antimicrobial, antioxidant and emulsifying properties of 19 Australian bushfoods and their products. Several bushfoods were found to possess strong antimicrobial activity against a number of common foodborne human pathogens and food spoilage microorganisms. A number of native plants have relatively strong antioxidant properties. Extracts from wattle seeds (*Acacia victoriae*) were found to have strong emulsifying capacities. These findings should provide a foundation for better marketing of bushfood products and expanding their areas of application.

Who is the report targeted at?
This report is primarily targeted at primary producers and processors of bushfoods. The information is also valuable to the hospitality and food processing industries which use native spices and flavours in their products. Furthermore, information on the emulsifying properties of wattle seed extracts has commercial potential and is especially targeted at food ingredient manufacturers who might be interested in commercialising the products. Finally, the information should also be of interest to the general consumers of bushfood products.

Background
The Australian bushfood industry has grown substantially since its establishment in the early 1980s. One of the main driving forces for the growth of the industry has been the appeal of its nativeness and a distinctively Australian flavour. While this image should be carefully preserved, it is believed that a sustainable growth strategy would require the industry to make significant inroads into the mainstream food markets by exploring alternative uses and markets for bushfoods beyond their traditional area of application - mainly as flavourings.

Many Australian bushfoods are known to possess functional properties such as antimicrobial, antioxidant and emulsifying properties. These properties have broad areas of application with significant commercial values. Most commercial antimicrobials, antioxidants and emulsifiers are synthetic compounds and as consumers are becoming increasingly sceptical about artificial food additives, natural substitutes are keenly sought after by the food industry. This presents a unique opportunity for value-adding and market expansion for the bushfood industry. However, knowledge in this area is rudimentary and obtained mainly through experience rather than through systematic, scientific investigation. This lack of systematic, commercially applicable knowledge is preventing the industry from realising these opportunities; research and development to bridge this knowledge gap would be extremely beneficial to the industry.

Aims and objectives
The overall aim of this project is to contribute to the development of the industry by enhancing our understanding of the functional properties of bushfoods. Specifically, the objectives of the project are:

1. to generate reliable data on the antimicrobial and antioxidant properties of bushfoods to enhance their consumer appeal;
2. to expand the application of bushfoods to areas including antimicrobials, antioxidants and emulsifiers for food applications to augment their current uses as flavouring agents; and
3. to develop antimicrobial, antioxidant and emulsifying agents for use as natural substitutes for their synthetic counterparts.

Methodology
Bushfood samples were provided by growers and processors located in East Gippsland, Victoria and Northern Rivers and Sydney, New South Wales. The samples were extracted with three solvents (hexane, water and methanol) with polarity varying from apolar to strongly polar. The extracts were sterilised by cold sterilisation techniques using sterile membrane filters with 0.45 and 0.22 μm pore size. The extracts were tested for activity against 29 common foodborne human pathogens and food spoilage bacteria, yeasts and moulds using the disc diffusion method.

Methanol extracts of the samples were tested for total phenolic content using the Folin-Ciocalteu procedure and for antioxidant activity using two methods: β-carotene bleaching and the DPPH free radical scavenging. Based on the initial results, five plants with relatively high antioxidant activities were selected for further analysis by the TEAC method and by high performance liquid chromatography (HPLC) to identify the active fractions.

Wattle seeds (*Acacia victoriae*) were ground and extracted sequentially with water, 70% ethanol and dilute alkaline solutions. The proteins in the extracts were isolated and analysed by a number of techniques including SDS-PAGE and capillary electrophoresis (CE) for molecular size and other properties. Aqueous extracts of wattle seeds were used to prepare food emulsions with varying proportions of fat, and the emulsions were tested for stability under varying conditions of processing and storage (pH, salt and temperature, etc.). The average particle size and distribution of the emulsions were analysed by small angle laser light scattering techniques.

Results and key findings
This project has generated a wealth of original data and made a number of significant findings that are relevant to the bushfood industry, the scientific community and the general consumers of bushfood products.

Many native plant food products were found to possess antimicrobial activity, several of which have relatively strong activities against a number of important foodborne human pathogens, food spoilage bacteria and yeasts. Mountain Pepper (*Tasmannia lanceolata*) berries and leaves were found to have the strongest antimicrobial activity, while eucalyptus oil is effective against the broadest spectrum of pathogens. All the other native plant products tested, with the exception of Warrigal greens, also had some degree of activity against some of the microorganisms, by either inhibiting or slowing down their growth. Thus, the findings of this study are able confirm the anecdotal evidence gathered by the industry in this respect, and the information can be used by the bushfood industry to promote their products.

Interestingly, some extracts, such as pepper berry and leaves, had strong activity against human pathogens and spoilage bacteria but had no activity against lactic acid bacteria. This information may prove very useful as lactic acid bacteria are beneficial microorganisms in a number of food products including cheese, yoghurt, and many fermented meat and vegetable products such as salami and sauerkraut. The selective antimicrobial activities of these extracts mean that, when used in these products, they can inhibit the pathogenic and spoilage bacteria while not affecting the normal fermentation process. All the native plant food products tested in this study were, however, ineffective against all the spoilage moulds studied.

All the native plants products tested were found to possess some antioxidant activity, however the activities varied considerably between plants. The activity, as determined by the β-carotene bleaching method, was highest in forest berry, followed by lemon aspen, Davidson plums and mountain pepper. The native plants also possess free radical scavenging activities, which also varied markedly among the plants. Native mint was found to have the greatest free radical scavenging activity, followed by lemon iron bark, quandong, forest berry, lemon myrtle and aniseed myrtle.

Most of the antioxidant activities were found in the methanol extract while little activity was found in the hexane extract. This suggested that the antioxidants were likely to be polar compounds.

Wattle seed extracts have strong emulsifying powers. Even at very low protein concentrations (0.17-1.12%), stable oil-in-water emulsions can be formed with lasting stability. For the purpose of comparison, milk, which is an emulsion stabilised primarily by milk proteins, contains more than 3% protein.

The stability of the wattle seed emulsions was affected by pH, with the emulsions being more stable at acidic than at neutral or alkaline pH values. It's worthwhile to note that many emulsion type food products, such as mayonnaise and salad dressings, are acidic, and wattle seed extracts could be used in these products. The emulsions are very heat stable and can withstand processing at temperatures as high as 115°C for 30 min.

Implications/Recommendations

Overall, wattle seed extracts appear to have a great potential as emulsifiers and stabilisers for the food industry, especially at low pH levels.

In conclusion, this project has confirmed some of the anecdotal evidence regarding the functional properties of Australian bushfoods. The relatively strong antimicrobial and antioxidant properties of some of the native plant products mean that they might be used in foods beyond their original purpose of being a flavouring agent. Wattle seed extracts could be a promising natural emulsifying agent applicable to a variety of food products.

1. Introduction

Although there is no conclusive statistics on the size of the Australian bushfood industry, some estimates have put the retail value of the industry at $20 million per annum (Phelps, 1997; Cherikoff, personal communication, 2001). This is a remarkable achievement considering that the industry only began from a standing start in the early 1980s. The growth of the industry is partly attributed to the appeal of its nativeness and a distinctively Australian flavour. It also owes a great deal to the persistence and passion of a number of the pioneers of the industry who saw the opportunity, appreciated the Australian character of the concept and built businesses from an un-utilised resource. Additionally, the industry has the potential to become a model agricultural industry in terms of agricultural sustainability, export potential and social responsibility. While this image should be carefully preserved, it is believed that a sustained growth strategy would require the industry to make significant inroads into the mainstream food markets by exploring alternative uses and markets for bushfoods beyond their traditional area of application - mainly as flavourings.

Many Australian bushfoods are known to possess functional properties such as antimicrobial, antioxidant and emulsifying properties. Lemon myrtle, for example, is known to have strong antimicrobial activity; wattle extracts possess emulsifying properties, particularly in dairy products, while many bushfoods are rich in antioxidant compounds. These properties are of significant commercial value with wide areas of application. Most commercial antimicrobials, antioxidants and emulsifiers are synthetic compounds and as consumers are becoming increasingly sceptical about artificial food additives, natural substitutes are keenly sought after by the food industry. This presents a unique opportunity that could be exploited by the bushfood industry. Exploitation of these functional properties could open up entirely new avenues for market growth. However, knowledge in this area is rudimentary and obtained mainly through experience rather than through systematic, scientific investigation. This lack of systematic, commercially applicable knowledge was confirmed during consultations with members of the industry, and research to improve knowledge in this area was seen to be extremely beneficial to the industry.

It is well recognised that with the current methods of food marketing, food additives are needed to provide diversity of food products. Antimicrobials are one of the most important classes of food additives and the continued introduction of new marketing techniques such as fresh, minimally processed and cooked-and-chilled foods means that interest in antimicrobials in the food industry will remain high (Branen, 1993). Most antimicrobial agents in commercial use are synthetic chemicals.

Emulsifiers are used widely in the food industry for the production of a wide range of emulsion-based foods including dairy, confectionery and bakery products. More than 500,000 metric tonnes of emulsifiers are produced and sold annually worldwide (Hasenhuettl, 1997). Like antimicrobial agents, most of these are synthetic compounds with only a small proportion being natural materials, including lecithin (mostly produced from soybeans) and proteins.

In recent years, nutritionists and the general public have come to regard foods as more than just sources of energy and essential nutrients. Certain minor components of foods are now recognised for their health-promoting properties. The antioxidant properties of many phytochemicals, in particular, have received considerable amount of attention, for their roles in alleviating the effects of some of the chronic diseases such as cardiovascular diseases and certain cancers. These health-benefiting effects have become powerful promotional tools for the food industry to market products to an increasingly health conscious society. In addition to their importance in diet, antioxidants also contribute to the stability of food and a number of antioxidants, again mostly synthetic compounds, are used for preventing the development

of oxidative rancidity and browning of a wide range of food products. As consumers are becoming increasingly sceptical about artificial food additives, their natural substitutes are keenly sought after by the food industry. This presents the bushfood industry with an opportunity to position some of its produce with antimicrobial, antioxidant or emulsifying properties as natural substitutes for their artificial counterparts.

It is, therefore, clear that exploitation of those functional properties could open up entirely new venues of application for bushfoods and create opportunities for development of new markets. However, a lack of commercially applicable knowledge in this area is preventing the industry from realising these opportunities and research and development to alleviate this deficiency would be extremely beneficial to the industry. Preliminary work carried out by researchers at UNSW (Forbes-Smith and Paton, 2002) has made a good started to bridge the gaps in knowledge in this area of research. The study presented in this report extends the range of microorganisms and bush foods studied and the emulsifying properties of wattle seed have been investigated here for the very first time.

Objectives

In light of the above discussion, the overall aim of this project is to build on the existing work and contribute to the development of the industry by enhancing our understanding of the functional properties of bushfoods. Specifically, the objectives of the project were:

1. to generate reliable data on the antimicrobial and antioxidant properties of bushfoods to enhance their consumer appeal;
2. to expand the application of bushfoods to areas including antimicrobials, antioxidants and emulsifiers for food applications to augment their current uses as flavouring agents; and
3. to develop antimicrobial, antioxidant and emulsifying agents for use as natural substitutes for their synthetic counterparts.

The outcomes of the project are expected to bring benefits to the industry in a number of ways, including:

- sustaining the health-benefiting claims of some of the bushfoods by credible scientific evidence;
- marketing bushfood products as natural substitutes for their artificial counterparts; and
- developing applications of bushfood products beyond the traditional area of flavourings.

The combined effect of these benefits is expected to be such that the industry will be able to add value to, and better market their products, thereby creating new growth opportunities.

2. Materials and Methods

2.1. Bushfoods

A total of 19 native plant foods were used in this study. Table 1 shows the common and species names of the plants. The native plant foods were initially supplied by Cherikoff Australian Ingredients, Sydney, New South Wales for the initial screening of their antimicrobial and antioxidant activities. In later experiments, native plant materials were also obtained from other sources, including Australian Rainforest Products, Northern Rivers, New South Wales, Tarnuk Bushfoods, East Gippsland, Victoria and Outback Bushfoods, Alice Springs, Northern Territory where the species and locations of the plants were identified, as detailed in later sections.

2.2. Screening of bushfoods for antimicrobial activity

2.2.1. Extraction of native plant bushfoods for screening of antimicrobial activity

Extractions were made on 18 of the native plant foods, while eucalyptus oil was supplied in liquid form. Extracts were prepared using three solvents separately: 100% hexane, water and 100% methanol, with polarity varying from apolar (hexane) to strongly polar (methanol). For each extraction, the bushfood sample was ground and mixed with the solvent in a 1:10 (w/w) ratio and the mixture was stirred with a magnetic stirrer continuously for 30 min at room temperature. The mixture was then centrifuged at 5,000*g* for 5 minutes and the supernatant was collected as the crude extract. The extracts were sterilised by cold sterilisation techniques using sterile membrane filters with 0.45 and 0.22 μm pore size. The extracts were tested for their antimicrobial activity using the methods described below. If not used immediately after extraction, the extracts were stored at - 20 °C until required.

The extracts were tested for activity against 11 pathogenic and seven spoilage bacteria, nine yeasts and two moulds. These microorganisms covered a wide spectrum of species that are most commonly encountered in the food industry. Details of the microorganisms are presented in Table 2. All microbial tests were conducted under aseptic conditions.

2.2.2. Culturing of microorganisms

Freeze-dried cultures of *Aeromonas hydrophila, Bacillus cereus, Escherichia coli* 0157*, Salmonella enteritidis, Shigella sonnei, Staphylococcus aureus, Vibrio cholerae, Acinetobacter baumannii, Bacillus subtilis, Pseudomonas aeruginosa* and *Psychrobacter phenylpyruvica* were reconstituted according to instructions of the culture supplier (Australian Collection of Microorganisms, The University of Queensland, Brisbane), and transferred onto nutrient agar plates. The plates were incubated at 30 °C for 24-48 hours and then either used immediately for subculturing or stored at 4 °C. The cultures were maintained by subculturing fortnightly.

For antimicrobial activity tests, the cultures were transferred from the agar plates to nutrient broth, which was then incubated at 30 °C with orbital shaking. The incubation was stopped when the cultures reached a cell concentration of $1x10^5$ cells/ml, which was measured by optical density. The actual incubation time varied with the bacterial species due to different growth rates. Minor adjustments of the cell concentration were made with sterile nutrient broth to ensure consistency in cell concentration in all the microbial cultures. The cell suspensions obtained were used in the antimicrobial activity tests described below.

Table 1 Native plant foods used in the current study

Species name	**Common name**
Acacia victroriae	Prickly Wattle
Acronychia acidula	Lemon Aspen
Anetholea anisata	Anisata/Aniseed Myrtle
Araucaria bidwillii	Bunya Nuts
Backhousia citriodora	Lemon Myrtle
Citrus glauca	Desert or Wild Lime
Davidsonia pruriens	Davidson Plum
Eucalyptus olida	Forest Berry
Eucalyptus stragiana	Lemon Ironbark
Mentha australis	River/Native Mint
Podocarpus elatus	Illawarra Plum
Prostanthera incisa	Cut Leaf Mintbush
Santalum acuminatum	Quandong
Solanum centrale	Bush Tomato
Syzygium luehmannii	Riberry
Tasmannia lanceolata	Mountain Pepper
Tasmannia lanceolata (fruit)	Pepper Berry
Tetragonia tetragonioides	Warrigal Greens
Eucalyptus spp.	Eucalyptus (oil)

Table 2 Species of microorganisms used in this study

Microorganism	Source
Pathogenic bacteria	
Aeromonas hydrophila	ACM[a]
Bacillus cereus	ACM
Campylobacter jejuni	ACM
Clostridium perfringens	ACM
Escherichia coli 0157	ACM
Listeria monocytogenes	ACM
Salmonella enteritidis	ACM
Shigella sonnei	ACM
Staphylococcus aureus	ACM
Vibrio cholerae	ACM
Yersinia enterocolitica	ACM
Spoilage bacteria	
Acinetobacter baumannii	ACM
Bacillus subtilis	Charles Sturt University Culture Collection[b]
Lactobacillus plantarum	Charles Sturt University Culture Collection[b]
Oenococcus oeni	Charles Sturt University Culture Collection[b]
Pediococcus cerevisiae	Charles Sturt University Culture Collection[b]
Pseudomonas aeruginosa	Charles Sturt University Culture Collection[b]
Psychrobacter phenylpyruvica	Charles Sturt University Culture Collection[b]
Yeasts	
Candida albicans	Charles Sturt University Culture Collection[b]
Candida. colliculosa	Charles Sturt University Culture Collection[b]
Candida lipolytica	Charles Sturt University Culture Collection[b]
Candida stellata	Charles Sturt University Culture Collection[b]
Hanseniaspora uvarum	UNSW Culture Collection[c]
Pichia anomala	UNSW Culture Collection
Pichia membranifaciens	UNSW Culture Collection
Rhodotorula mucilaginosa	UNSW Culture Collection
Schizosaccharomyces octosporus	UNSW Culture Collection
Moulds	
Monilia fructicola	Charles Sturt University Culture Collection[d]
Botrytis cinerea	Charles Sturt University Culture Collection[d]

[a]Purchased from the Australian Collection of Microorganisms (ACM), The University of Queensland.
[b]Obtained from the School of Agriculture Microbiology Laboratory, Charles Sturt University.
[c]Provided by Professor Graham Fleet, School of Chemical Engineering and Industrial Chemistry, University of New South Wales.
[d]Obtained from the School of Wine and Food Sciences, Charles Sturt University.

Cultures of the three lactic acid bacteria, *Lactobacillus plantarum, Oenococcus oeni* and *Pediococcus cerevisiae* were maintained on MRS agar (Oxoid CM0361) and cell suspensions were obtained by subculturing in MRS broth (Oxoid CM0359), which were incubated at 30 °C. *Campylobacter jejuni* was maintained on blood agar (Oxoid CM0271) supplemented with 7% horse blood (Oxiod SR0048) and subcultured in tryptone soy broth (Oxoid CM0129), which was incubated at 37 °C. *Clostridium perfringens* was maintained in cooked-meat medium at 4 °C and subcultured in tryptic soy broth. *Listeria monocytogenes* and *Yersinia enterocolitica* were maintained on tryptic soy agar and subcultured in tryptone soy broth, which was incubated at 30 °C.

All the yeast cultures were maintained on potato dextrose agar (Oxiod CM0139) and subcultured in potato dextrose broth, which was incubated at 25 °C. All the broth cultures were incubated with orbital shaking until a cell concentration of $1x10^5$ cells/ml was reached.

2.2.3. Assay of antimicrobial activities

Each of the microbial cultures, obtained as described above, were tested against the three different extracts (hexane, water and methanol) of each bushfood. For each test, 1 ml of a microbial culture was added to a Petri dish containing 15 ml of the same broth medium (kept at 45 °C) that it was cultured in. The medium was mixed thoroughly by slow rotation and allowed to solidify. A 0.1 ml aliquot of each bushfood extracts was pipetted onto a plain antibiotic test disc (3 mm in diameter), which was then placed on the solidified agar. Six discs were usually placed in one Petri dish. Control discs were prepared exactly the same way except that the extracts were substituted by the respective solvents. The agar plates were then incubated at the optimal growth temperatures of respective microorganisms as described above for 48 h. After this, the plates were covered by microbial growth except in the clear zones surrounding the discs where no microbial growth had occurred due to the antimicrobial activity of the extracts. The sizes of the clear zones were then determined, and served as a measure of the antimicrobial activity of the extracts.

The antifungal activity was tested differently. The mould cultures were maintained on potato dextrose agar and subcultured onto the same agar medium, which was incubated at 25 °C for 72 hours. After which, a piece of the agar block, 4x4 mm in size, containing fungal mycelium, was cut and placed onto potato dextrose agar containing 1% of the bushfood extracts. The agar plates were then incubated at 25 °C for 72 h and the size of the fungal growth was measured.

2.3. Screening of bushfoods for antioxidant activity

2.3.1. Extraction of bushfoods for initial screening of antioxidant activity

Samples of each bushfood (10g dry matter) were ground and mixed with 50 ml methanol in a beaker and the mixture was stirred with a magnetic stirrer continuously for 2 hours. The mixture was then filtered with Whatman No. 1 paper and the plant residue was washed with an extra 20 ml of methanol to ensure complete extraction. Filtrates from the extraction were collected and concentrated under a fume hood to a final standard volume of 32 ml, and were analysed for antioxidant activity by three different methods.

2.3.2. Determination of total phenolic content of plant extracts (Folin-Ciocalteu method)

The plant extracts were diluted to 1% (w/v) dry matter with methanol and then 1 ml aliquots of the diluted extracts were mixed with 0.5ml of Folin-Ciocalteu phenol reagent and 3.0ml of 20% Na_2CO_3. This mixture was vortexed immediately and left to stand at room temperature

for 15 minutes. After this, the mixture was diluted with 10ml of deionized water, which was then centrifuged at 2,500*g* for 5 min. The absorbance of the supernatant was read with a Unicam 8625 UV/Vis Spectrophotometer at 725nm. Methanol was used as a control in place of the plant extract. A standard curve of gallic acid was prepared with concentrations of 20, 40, 60, 80, and 100 mg/L. The total phenolic content of each plant extract was calculated from the standard curve and expressed as gallic acid equivalent in (GAE) mg/L.

2.3.3. Assay of antioxidant activity (β-carotene bleaching method)

In this method, a solution containing 1mg β-carotene and 10 mL chloroform was made. 1.5mL of the solution was well mixed with 20 mg linoleic acid and 200 mg Tween 80 to make an emulsion, and then nitrogen gas was pumped through to remove chloroform. After this, the emulsion was diluted with 100 ml of oxygenated deionized water which was prepared by bubbling air through for 5 minutes. A 3 ml aliquot of the β-carotene/linoleic acid emulsion was then mixed with 40 µl of the plant extract in a test tube and the absorbance at time 0 min was taken immediately on a Unicam 8625 UV/Vis Spectrophotometer at 470nm. The emulsion was then incubated in a water bath set at 50°C for 60 min and the absorbance was taken again. A control was prepared with the same procedure except that the plant extract was replaced with methanol. The antioxidant activity, expressed as percent inhibition of the bleaching of β-carotene relative to the control containing no antioxidant, was calculated using the following equation:

$$AOA = 100(DR_c - DR_s) / DR_c$$

where
AOA = antioxidant activity (% inhibition of bleaching)
DR_c = degradation rate of control
= ln (a/b) / 60
DR_s = degradation rate of sample
= ln (a/b) / 60
a = absorbance at time 0 min
b = absorbance at time 60 min

2.3.4. Assay of free radical scavenging activity (DPPH method)

In this method, 1 ml of plant extract, previously diluted to 0.01% (w/v) of original plant dry matter, was mixed with 1 m of 0.1mM 1,1-diphenyl-2-picrylhydraxyl (DPPH) free radicals. The reaction mixture was vortexed for 3 s. Absorbance was determined immediately using a Unicam 8625 UV/Vis spectrophotometer at 520nm, with methanol used as the blank. Absorbance was determined again at time 2 h. A control was prepared by substituting the plant extract with methanol. Free radical scavenging activity (%SA) was calculated using the following equation: %SA = (1- absorbance in the presence of sample/ absorbance in the absence of sample) x 100. All analyses were done in triplicates.

2.4. Detailed analysis of antioxidant activity of selected native plants

Five species with relatively high antioxidant activities were selected for further analysis.

2.4.1. Soxhlet extraction of plant leaves

Leaves of *Eucalyptus olida* GL, *Mentha australi*s GL and *Tasmannia lanceolata* GL were obtained from Tarnuk Bushfoods (East Gipsland, Vic), dried and ground as is commercially available. *Backhousia citriodora* NR, *Eucalyptus stragiana* NR and *Eucalyptus olida* NR

were obtained fresh from Australian Rainforest Products (Northern Rivers, NSW). These were dried and ground according to the methods as used at Tarnuk Bushfoods.

Three soxhlets were set up with approximately 8 g of each plant product. Each sample was extracted sequentially with hexane, ethyl acetate and methanol and 10 cycles were run for each solvent with the residue carried over in succession. This produced three replicates for each of the three solvents. The first two of these replicates were pooled and dried in a rotary evaporator. The third was subjected to a liquid/liquid partition with hexane/methanol used for the hexane and methanol extracts and ethyl acetate/water for the ethyl acetate extracts. These were separated and dried by rotary evaporation.

This process provided nine extracts and partitions (fractions) per plant: hexane crude extract, ethyl acetate crude, methanol crude, hexane - methanol soluble, hexane - hexane soluble, ethyl acetate - ethyl acetate soluble, ethyl acetate - water soluble, methanol - hexane soluble, methanol - methanol soluble. Each extract or fraction was analysed for antioxidant activity.

2.4.2. Assay of antioxidant activity (TEAC method)

A 5 µl aliquot of each extract was pipetted in quadruplicate into a 96 well microplate. To each well 100 µl of 2.2 mM ABTS solution was added followed by 100µl of 1 mM potassium persulfate solution. Absorbency for the plate was read at 734 nm every minute for 1 hour. TROLOX and quercetin solutions (0, 0.3, 0.7, 1.4, 2.0, 2.5 mM) were prepared as antioxidant standards. TEAC and QEAC values were calculated using a curve generated from the TROLOX and quercetin standards respectively.

2.4.3. Reversed phase high performance liquid chromatography (HPLC)

Crude extracts and fractions of native plants were analysed using a Waters Alliance HPLC with a 2996 Photodiode Array detector. The HPLC column used was a Phenomonex Luna $C18_{(2)}$ unit (5µ, 250x4.6, 100Å) (Waters, Lane Cove, Australia). Separation was achieved using gradient elution with the solvents being 0.5% aqueous acetic acid (Solvent A) and acetonitrile (Solvent B). The elution gradient used was from 0% B to 30% B in 25 minutes, held isocratic for 10 minutes then increased from 30% B to 100% B in 20 minutes. The solvent was held isocratic at 100% B for a further 5 minutes before being returned to 0% B in 5 minutes and held at 0% B for 10 minutes before the next injection. Flow rate was set to 1 ml/minute.

2.4.4. Post-column reaction system for detection of active antioxidants

To elucidate which of the compounds separated by HPLC were active antioxidants the eluent was reacted 'online' with the free radical chromogen ABTS (Figure 1). The ABTS free radical (ABTS) was prepared by adding 5 ml of potassium persulphate (24.5 mM) to 45ml of ABTS (7 mM). This was left to develop for at least 12 hours. 35 ml of the prepared ABTS solution was added to 2 L of Milli Q water. Using a Waters Reagent Manager the ABTS solution was introduced to the Post-column HPLC flow via a PEEK t-piece at a flow rate of 0.5 ml/minutes and absorbance detected at 415 nm on a Waters 482 UV/Vis detector. When the Alliance HPLC was delivering solvent only, the recorded absorbance on the W482 was ~0.6 AU. The data from the W482 was digitised using the Waters Sat/IN module to allow for integration of the antioxidant troughs (reverse peaks). TEAC and QEAC values were calculated for each individual peak using a standard curve generated from injection of the standard TROLOX and quercetin solutions in increasing concentrations.

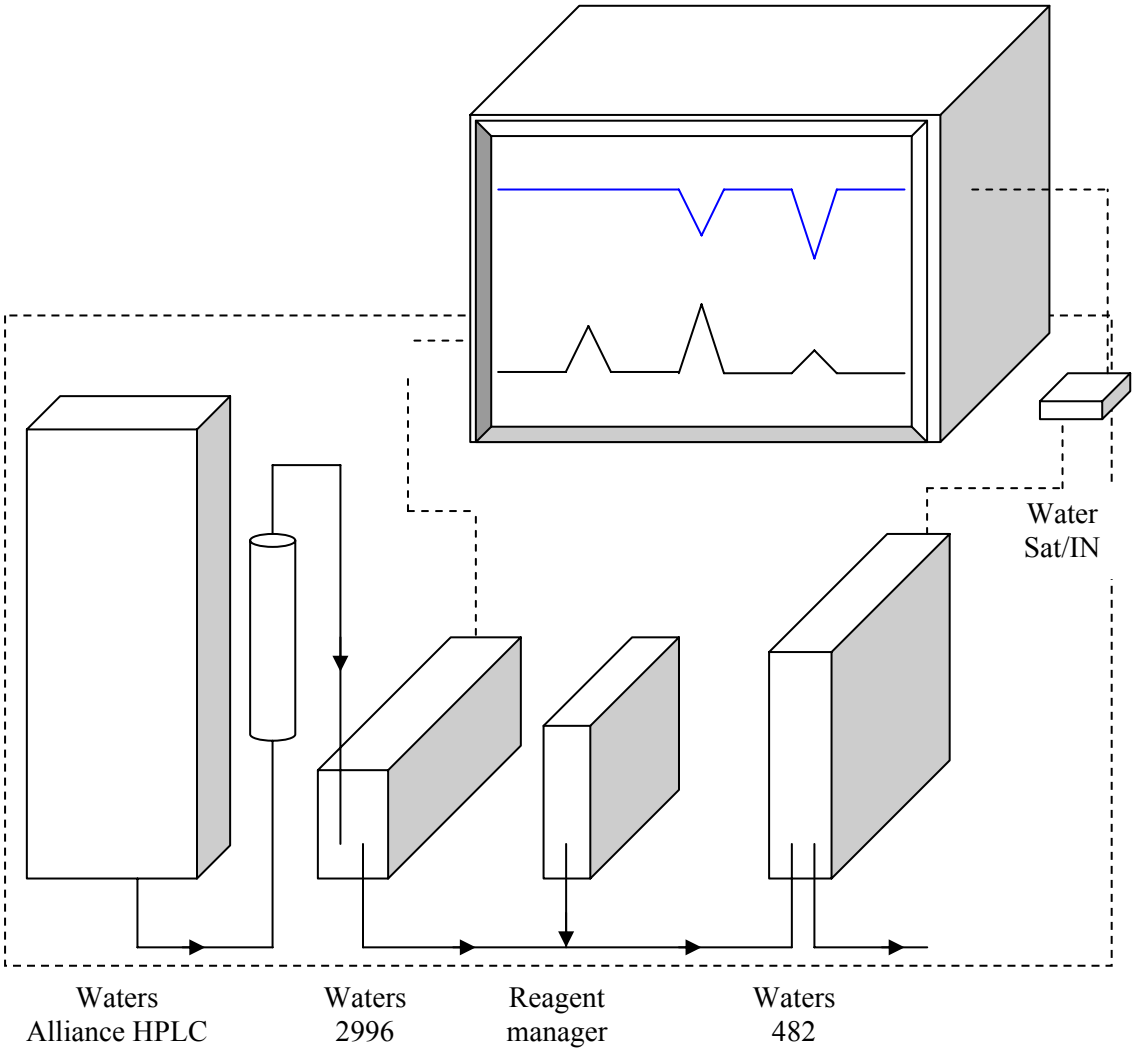


Figure 1. A schematic representation of the HPLC and post-column reaction systems for detecting active antioxidant peaks in extracts of native plant products

2.5. Emulsifying properties of wattle seed extracts

Whole wattle seeds (*Acacia victoriae*) were supplied by Outback Bushfoods, Alice Springs, Australia. Canola oil was purchased from the local Woolworths supermarket. All other reagents and chemicals were supplied by Sigma-Aldrich, Castle Hill, New South Wales, Australia.

2.5.1. Extraction of wattle seed flours

Whole wattle seeds or the de-hulled cotyledons (inside portion) were milled separately to pass through a 0.11 mm mesh and then extracted sequentially with distilled water, 0.1 M NaOH solution and 70 % ethanol. Each flour was extracted with ten times the volume of solvent three times, stirring for one hour each time before centrifuging at 3000*g* to separate the

residue from the soluble portion (supernatant), after which the supernatants for each extracting solvent were pooled. The alkali fraction was further purified by isoelectric precipitation at pH 3.85. All fractions were freeze dried using a Christ-Alpha 1-4 Freeze dryer (Biotech International, Germany).

2.5.2. Characterisation of wattle seed extracts

The extracts were characterised by yield, proximate analysis, SDS-PAGE and CE profiles. The total yield was obtained by weighing the freeze-dried isolates while the proximate analysis was determined by standard AOAC (1996) methods. SDS-PAGE analysis of the protein isolates was determined by using the Laemmli buffer system (Laemmli, 1970) and running the samples on a pre-cast gel (4-20 % gradient) with a Bio-Rad Mini PROTEAN® 3 system (Bio-Rad Laboratories, Hercules, CA, USA). The bands were stained with Coomassie brilliant blue R-250. Molecular weight markers ranging from 6.5 to 205 kDa were also run on the same gel and the results were used to estimate the molecular weight profile of the protein bands from the stained gels using the Genetools™ software. The CE profile was determined on Beckman P/ACE system 5510 (Beckman Coulter Inc., CA, USA) according to the method of Basha (1997). The electrophoresis was conducted at 25°C using an uncoated fused-silica capillary of 75 μm internal diameter and 57 cm total length. Samples were dissolved in 0.01 M sodium phosphate buffer (pH 8.3) and injected by pressure for 30 seconds. Each separation run was for 10 minutes at 25 kV voltage in 0.3 % sodium tetraborate buffer while the detector was set at 214 nm. The capillary was rinsed sequentially between successive electrophoretic runs with 0.1 M NaOH (2 minutes), 1 M HCl ((1 minute), distilled water (2 minutes) and the run buffer (2 minutes). Data were processed with the P/ACE system 5000 series software.

2.5.3. Investigation of emulsifying properties of wattle seed extracts

A typical oil-in-water emulsion was prepared using canola oil as the dispersed phase and the appropriate amount of supernatant of whole wattle seed extract in 0.01 M phosphate buffer (pH adjusted by either 1 M NaOH or 1M HCl) as the continuous phase. After mixing the oil with the volume of protein solution to give the appropriate oil contents of 20%, 50% and 80% (v/v) respectively in the final emulsion, the coarse emulsion was passed at no pressure through a two-stage high pressure homogeniser (Niro Soavi, Parma, Italy) to form a pre-emulsion. Then, using a constant first stage pressure of 14.3 MPa, the pressure was increased to 28.6 MPa using the second stage control valve after which the sample was collected. Each emulsion was passed three times through the homogeniser, to ensure complete dispersion of the oil. Based on measured values of solids and protein contents of the wattle seed extract, the 20%, 50% and 80% oil-in-water emulsions contained 1.1%, 0.43% and 0.17% (w/v) protein contents respectively.

The effect of salt on the emulsions was studied in the 20% and 50% oil-in-water emulsions prepared at pHs 4 and 5 by adding the appropriate amounts of NaCl to the water (buffer) phase to obtain 0.25, 0.5 and 1% (w/v) concentration in the final emulsion. The 20% emulsions were also sterilised at 115°C for 30 minutes in a vertical retort in order to study their stability to heat.

2.5.4. Characterisation of emulsions

The effect of storage temperature on all emulsions was studied by dividing the emulsions into two groups and storing at either refrigeration temperature (5°C) or in an incubator at 25°C for up to seven days. Each preparation also had 0.05% (w/v) sodium azide added to prevent microbial contamination. Every day, the emulsions were visually examined for signs of creaming, oiling off or other physical separation attributes. Furthermore, the droplet size distribution and hence, the weight-volume average emulsion particle diameter (d_{43}), defined

as $\Sigma N_i d_i^4 / \Sigma N_i d_i^3$, were measured by small angle laser light scattering using a Mastersizer E (Malvern Instruments Ltd, Worcestershire, England). The presentation factor was 0303 (i.e. refractive index of 1.414 and absorption of 0.001) and a polydisperse model was chosen for the size distribution using a lens of 45 mm focal length. Emulsion droplets were sized using distilled water as the dispersant (Agboola *et al.*, 1998).

2.5.5. Measurement of viscosity

The viscosity of the wattle seed extract and the emulsions formed with each extract at different pH values were measured using a Brookfield viscometer. These measurements were made on the same day of extraction or emulsion preparation in triplicates and average values were reported.

2.6. Statistical analysis

All extractions and analyses were carried out at least in triplicates and the means reported. Data collected were subjected to analysis of variance, and means of treatments showing significant difference ($P < 0.05$) were subjected to Fisher's least significant difference test.

3. Results and Discussion

3.1. Antimicrobial properties of Australia bushfoods

3.1.1. Activity against common foodborne human pathogens

The antimicrobial activities of methanol, water and hexane extracts of Australian native plant foods are presented in Tables 3-5, respectively. All the native plant extracts, with the exception of Warrigal greens, showed some degrees of activity against pathogenic bacteria, by either completely inhibiting or slowing down the microbial growth. Furthermore, several extracts exhibited relatively strong activities against a number of important pathogens. The strongest activity was shown by the extracts of pepper leaves and pepper berry, followed by riberry, wild lime, native mint and eucalyptus oil. Davidson plum, lemon aspen, lemon ironbark, cut leaf mintbush and lemon myrtle also exhibited various degrees of activity while the activities of the remaining native plant extracts were relatively minor.

While pepper leaves gave extracts with the strongest activities, pepper berry and eucalyptus oil showed inhibitory activity against the broadest spectrum of pathogens (Table 3). Both plant extracts exhibited activity against all the eleven foodborne human pathogens tested with the strongest activity shown against the cholera-causing bacterium *Vibrio cholerae*. Pepper berry also gave extracts with very strong activities against *Staphylococcus aureus*, *Clostridium perfringens* and *Aeromonas hydrophila*, the former two, in particular, having been frequently implicated in food poisoning outbreaks (Stewart, 2003; Bates and Bodnaruk, 2003).

Of the three types of extraction solvents used, methanol produced extracts with the strongest and broadest activities by far, while water appeared to be the least efficient medium in extracting antimicrobial materials.

3.1.2. Activity against common food spoilage bacteria

The activities of Australian native plant extracts against seven of the common food spoilage bacteria are presented in Tables 3-5. Again, with the exception of Warrigal greens, all the native plant extracts showed some degree of activity against the bacteria, by either completely inhibiting or slowing down the microbial growth. Strongest activity was shown by the extracts of pepper berry and leaves, followed by aniseed myrtle, quandong, eucalyptus oil and wild lime. Extracts of lemon myrtle, bush tomatoes, forest berry, lemon ironbark, lemon aspen and Davidson plum also showed an intermediate degree of activity, while the activities of the remaining plant extracts were relatively insignificant.

While the native pepper leaves and berries gave extracts with the highest activity, eucalyptus oil showed inhibitory activity against the broadest spectrum of food spoilage bacteria. The eucalyptus oil exhibited antimicrobial activity against all seven bacterial species tested, with the strongest activity being against *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Bacillus subtilis*.

Table 3. Antimicrobial activity of methanol extract of Australian native plants

	Lemon myrtle	Pepper berry	Bush tomato	Pepper leaf	Native mint	Forest berry	Anisata	Lemon ironbark	Warrigal greens	P. incisia	Aniseed myrtle	Illawarra plums	Wild limes	Lemon aspen	Davidson plums	Bunya nuts	Quandong	Riberry
Pathogenic bacteria																		
Aeromonas hydrophila	7.5 [a]	12.0 [a]	n.a	12.0 [a]	8.0 [b]	8.5 [b]	4.7 [c]	8.0 [a]	n.a	9.0 [b]	n.a	n.a	8.0 [a]	7.0 [b]	10.0 [a]	n.a	8.0 [a]	8.0 [a]
Bacillus cereus	7.7 [b]	11.2 [a]	8.0 [b]	12.8 [a]	7.8 [a]	7.8 [a]	2.3 [c]	7.2 [b]	n.a	7.2 [a]	n.a	4.3 [c]	9.0 [a]	12.0 [a]	11.7 [a]	n.a	8.0 [a]	9.3 [a]
Campylobacter jejuni	n.a	3.4 [c]	3.2 [c]	n.a	n.a	2.8 [b]	n.a	n.a	n.a	1.8	n.a	4.0 [b]	8.0 [b]	n.a	n.a	n.a	n.a	n.a
Clostridium perfringens	2.3 [b]	13.0 [b]	n.a	10.7 [b]	11.7 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.0 [a]	11.3 [b]	12.7 [b]	n.a	12.0 [c]	14.7 [b]
Escherichia coli 0157:H7	n.a	7.3 [b]	n.a	8.0 [b]	n.a	7.7 [b]	n.a	6.8 [b]	n.a	n.a	n.a	n.a	7.0 [c]	n.a	7.3 [c]	n.a	6.0 [c]	n.a
Listeria monocytogenes	5.7 [b]	12.5 [b]	6.2 [b]	15.8 [b]	13.6 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	0.0	7.4 [a]	n.a	n.a	n.a	4.8 [a]
Shigella sonnei	n.a	7.7 [c]	9.0 [c]	8.3 [c]	n.a	8.3 [b]	n.a	4.5 [b]	n.a	n.a	n.a	n.a	8.0 [c]	n.a	2.3 [c]	n.a	n.a	n.a
Staphylococcus aureus	n.a	14.3 [a]	n.a	14.5 [a]	6.8 [a]	8.7 [b]	n.a	5.0 [b]	n.a	8.0	n.a	n.a	n.a	7.7 [b]	7.0 [c]	n.a	7.0 [c]	8.7 [a]
Salmonella enteritidis	n.a	n.a	n.a	n.a	n.a	6.5 [c]	n.a	2.2 [c]	n.a	n.a	n.a	n.a	7.0 [a]	n.a	n.a	n.a	n.a	n.a
Vibrio cholerae	9.7 [a]	15.0 [a]	9.7 [a]	14.7 [a]	9.7 [a]	12.3 [a]	7.8 [a]	11.7 [a]	n.a	9.7 [a]	7.7 [b]	7.0 [a]	12.0 [a]	9.0 [a]	12.7 [a]	n.a	9.7 [a]	8.0 [a]
Yersinia enterocolitica	n.a	8.7 [b]	n.a	7.3 [a]	n.a	9.0 [b]	7.5 [b]	7.5 [b]	n.a	n.a	n.a	n.a	14.0 [c]	n.a	7.8 [b]	n.a	7.0 [c]	8.3 [b]
Spoilage bacteria																		
Acinetobacter baumannii	8.0 [b]	8.0 [b]	10.0 [c]	9.7 [b]	8.0 [b]	9.3 [a]	n.a	8.3 [a]	n.a	9.0 [a]	n.a	n.a	7.2 [b]	8.7 [b]	10.7 [a]	n.a	10.7 [b]	7.5 [b]
Bacillus subtilis	4.0 [b]	17.0 [a]	3.3 [c]	16.0 [a]	7.0 [c]	6.5 [c]	n.a	n.a	n.a	6.5 [c]	14.3 [b]	n.a	2.3 [c]	6.8 [a]	7.3 [a]	n.a	9.0 [c]	7.3 [c]
Lactobacillus plantarum	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Oenococcus oeni	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Pediococcus cerevisiae	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Pseudomonas aeruginosa	7.5 [a]	10.8 [a]	7.0 [a]	10.3 [a]	1.8	12.0 [b]	n.a	8.3 [b]	n.a	n.a	n.a	n.a	7.0 [a]	7.7 [b]	6.8 [b]	n.a	7.7 [c]	7.3 [a]
Psychrobacter phenylpyruvica	n.a	8.0 [a]	4.5 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	4.3 [c]	6.5 [a]	6.8 [a]	n.a	n.a	n.a
Spoilage yeasts																		
Candida albicans	n.a	11.8 [a]	n.a	10.8 [a]	n.a	8.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Candida colliculosa	1.8	18.8 [a]	n.a	17.8 [a]	n.a	7.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Candida lipolytica	2.3	10.3 [b]	n.a	10.3 [b]	n.a	7.7 [a]	n.a	2.7	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Candida stellata	n.a	20.0 [a]	n.a	16.5 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Hanseniaspora uvarum	1.6	16.5 [a]	n.a	15.5 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Pichia anomala	n.a	10.0 [a]	n.a	10.3 [a]	n.a	4.8 [b]	n.a	4.3 [c]	4.3 [c]	4.3 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Pichia membranifaciens	7.3 [c]	10.3 [a]	n.a	10.0 [a]	n.a	n.a	n.a	n.a	n.a	10.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Rhodotorula mucilaginosa	7.7 [b]	17.0 [a]	n.a	15.0 [a]	n.a	12.0 [b]	n.a	9.0 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Schizosaccharomyces octosporus	9.3 [b]	34.7 [a]	n.a	34.7 [a]	5.5 [c]	7.0 [c]	n.a	6.0	n.a	9.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a

Key to Table: The results are obtained by the agar disc diffusion method. The data were means of at least three replicate tests and are expressed as the area (mm in diameter) in which microbial growth was either completely inhibited or slowed down. [a] Microbial growth completely inhibited; [b] growth slowed down markedly; [c] growth somewhat affected; n.a. no activity.

Table 4. Antimicrobial activity of water extract of Australian native plants

	Lemon myrtle	Pepper berry	Bush tomato	Pepper leaf	Native mint	Forest berry	Anisata	Lemon ironbark	Warrigal greens	P. incisia	Aniseed myrtle	Illawarra plums	Wild limes	Wattle	Eucalyptus oil	Lemon aspen	Davidson plums	Bunya nuts	Quandong	Ribberies
Pathogenic bacteria																				
Aeromonas hydrophila	7.5	8.3 [b]	n.a	6.8 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	4.5 [c]	7.7 [a]	10.0 [a]	8.0 [c]	7.7 [b]	n.a	8.0 [c]	n.a
Bacillus cereus	n.a	9.0 [a]	7.3 [b]	8.0 [b]	n.a	6.7 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	6.8 [a]	9.0 [a]	7.5 [a]	7.7 [a]	n.a	6.8 [a]	6.7 [a]
Campylobacter jejuni	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.8 [a]	n.a	n.a	n.a	n.a	n.a
Clostridium perfringens	5.0 [c]	2.7 [c]	7.0 [c]	3.3 [c]	7.0 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.0 [a]	6.3 [c]	10.7 [c]	2.7 [c]	n.a	n.a
Escherichia coli 0157:H7	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	7.3 [c]	9.7 [a]	n.a	n.a	n.a	n.a	n.a
Listeria monocytogenes	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	7.3 [a]	n.a	n.a	n.a	n.a	n.a
Shigella sonnei	n.a	n.a	8.0 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	9.0 [a]	n.a	2.3 [c]	n.a	n.a	n.a
Staphylococcus aureus	n.a	7.3 [a]	n.a	7.3 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	7.3 [a]	n.a	n.a	n.a	n.a	n.a
Salmonella enteritidis	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	7.5 [a]	n.a	n.a	n.a	n.a	n.a
Vibrio cholerae	n.a	9.3 [a]	7.3 [b]	8.7 [a]	2.2 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a [c]	10.7 [a]	13.3 [a]	7.2 [b]	8.0 [a]	n.a	6.5 [b]	6.5 [b]
Yersinia enterocolitica	n.a	n.a	n.a	n.a	n.a	n.a	7.5 [b]	n.a	n.a	n.a	n.a	n.a	n.a [c]	n.a	11.3 [a]	n.a	n.a	n.a	n.a	n.a
Spoilage bacteria																				
Acinetobacter baumannii	n.a	n.a	9.7 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	9.3 [c]	7.5 [b]	10.3 [a]	7.7 [c]	11.0 [c]	n.a	9.0 [c]	7.7 [c]
Bacillus subtilis	10.0 [b]	19.0 [a]	7.5 [a]	16.0 [a]	7.0 [a]	10.3 [b]	14.7 [a]	7.7 [b]	n.a	n.a	18.0 [a]	n.a	3.3 [c]	7.0 [b]	10.0 [a]	8.0 [c]	7.0 [a]	n.a	8.0 [c]	n.a
Lactobacillus plantarum	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	9.0 [a]	n.a	n.a	n.a	n.a	n.a
Oenococcus oeni	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.7 [a]	n.a	n.a	n.a	13.5 [c]	n.a
Pediococcus cerevisiae	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.3 [c]	n.a	n.a	n.a	n.a	n.a
Pseudomonas aeruginosa	7.0 [a]	11.7 [a]	5.3 [c]	10.3 [a]	5.0 [c]	7.7 [b]	n.a	7.0 [a]	n.a	n.a	n.a	n.a	8.7 [b]	6.7 [c]	11.0 [a]	7.0 [a]	7.0 [a]	n.a	8.0 [c]	8.0 [c]
Psychrobacter phenylpyruvica	n.a	n.a	n.a	n.a	2.7 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	6.5 [a]	2.3 [c]	3.0 [c]	n.a	2.7 [c]	2.3 [c]
Spoilage yeasts																				
Candida albicans	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.3 [a]	n.a	n.a	n.a	n.a	n.a
Candida colliculosa	n.a	9.0 [b]	n.a	8.8 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	9.0 [a]	n.a	n.a	n.a	n.a	n.a
Candida lipolytica	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.7 [a]	n.a	n.a	n.a	n.a	n.a
Candida stellata	n.a	10.8 [b]	n.a	9.3 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	9.0 [a]	n.a	n.a	n.a	n.a	n.a
Hanseniaspora uvarum	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	9.3 [a]	n.a	n.a	n.a	n.a	n.a
Pichia anomala	n.a	7.3 [a]	n.a	6.5 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.0 [a]	n.a	n.a	n.a	n.a	n.a
Pichia membranifaciens	n.a	7.0 [c]	n.a	7.0 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.0 [b]	n.a	n.a	n.a	n.a	n.a
Rhodotorula mucilaginosa	n.a	9.0 [b]	n.a	8.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	9.0 [a]	n.a	n.a	n.a	n.a	n.a
Schizosaccharomyces octosporus	0.8	23.5 [a]	n.a	21.5 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	12.0 [a]	n.a	n.a	n.a	n.a	n.a

Key to Table: The results are obtained by the agar disc diffusion method. The data were means of at least three replicate tests and are expressed as the area (mm in diameter) in which microbial growth was either completely inhibited or slowed down. [a] Microbial growth completely inhibited; [b] growth slowed down markedly; [c] growth somewhat affected; n.a. no activity.

Table 5. Antimicrobial activity of hexane extract of Australian native plants

	Lemon myrtle	Pepper berry	Bush tomato	Pepper leaf	Native mint	Forest berry	Anisata	Lemon ironbark	Warrigal greens	P. incisia	Aniseed myrtle	Illawarra plums	Wild limes	Lemon aspen	Davidson plums	Bunya nuts	Quandong	Ribberies
Pathogenic bacteria																		
Aeromonas hydrophila	7.5 [a]	13.0 [a]	n.a	11.0 [a]	n.a	10.7 [a]	n.a	9.0 [b]	n.a	8.3 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Bacillus cereus	9.0 [a]	13.0 [a]	n.a	12.3 [a]	8.0 [b]	6.8 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.5 [a]	n.a	2.3 [b]	n.a	7.5 [a]
Campylobacter jejuni	4.2 [c]	8.3 [c]	5.5 [c]	n.a	n.a	3.1 [b]	n.a	1.4	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Clostridium perfringens	n.a	13.0 [b]	n.a	9.0 [a]	6.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	9.7 [b]	n.a	n.a	2.7 [c]	15.0 [b]
Escherichia coli 0157:H7	n.a	8.0 [b]	n.a	7.0 [b]	n.a	8.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Listeria monocytogenes	1.4 [b]	3.8 [b]	n.a	4.2 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	4.7 [a]	n.a	n.a	n.a	4.2 [a]
Shigella sonnei	0.0	8.3 [a]	n.a	7.3 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Staphylococcus aureus	6.7 [a]	15.3 [a]	n.a	13.7 [a]	4.3 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	7.3 [a]	n.a	n.a	n.a	10.0 [a]
Salmonella enteritidis	2.2 [c]	6.5 [c]	n.a	4.3 [c]	n.a	6.5 [c]	n.a	7.0 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Vibrio cholerae	10.7 [a]	15.7 [a]	7.0 [b]	13.0 [a]	8.3 [a]	8.5 [a]	n.a	8.3 [a]	n.a	7.5 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Yersinia enterocolitica	8.0 [b]	9.7 [a]	n.a	8.7 [b]	n.a	14.0 [b]	n.a	11.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Spoilage bacteria																		
Acinetobacter baumannii	10.0 [a]	9.0 [a]	3.0 [c]	7.8 [b]	8.7 [b]	8.3 [b]	n.a	7.8 [c]	n.a	10.7 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Bacillus subtilis	7.5 [b]	16.0 [a]	3.5 [c]	14.0 [a]	n.a	8.0 [b]	13.0 [a]	9.5 [a]	n.a	7.3 [b]	n.a	n.a	n.a	n.a	n.a	n.a	7.0 [c]	n.a
Lactobacillus plantarum	n.a	9.0 [b]	n.a	8.0 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	5.0 [c]	n.a	n.a	n.a	n.a	n.a	n.a
Oenococcus oeni	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.3 [c]	n.a	10.0 [c]	n.a	n.a
Pediococcus cerevisiae	n.a	8.0 [b]	n.a	6.8 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Pseudomonas aeruginosa	11.0 [a]	10.0 [a]	n.a	9.0 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	8.0 [a]	n.a	n.a	n.a	9.3 [a]
Psychrobacter phenylpyruvica	6.7 [a]	8.7 [a]	n.a	6.5 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Spoilage yeasts																		
Candida albicans	1.8	12.0 [a]	n.a	9.8 [a]	n.a	8.7 [b]	n.a	8.3 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Candida colliculosa	9.8 [b]	19.0 [a]	n.a	17.5 [a]	n.a	7.0 [b]	n.a	2.7	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Candida lipolytica	8.0 [c]	10.7 [b]	n.a	10.7 [b]	n.a	7.0 [a]	n.a	7.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Candida stellata	3.8 [c]	17.8 [a]	9.5 [c]	17.8 [a]	n.a	7.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Hanseniaspora uvarum	7.8 [a]	16.8 [a]	n.a	14.8 [a]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Pichia anomala	7.3 [c]	11.0 [a]	n.a	9.7 [a]	n.a	9.0 [b]	n.a	7.0 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Pichia membranifaciens	9.8 [b]	11.0 [a]	n.a	8.7 [a]	n.a	10.3 [b]	n.a	n.a	n.a	7.0 [c]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Rhodotorula mucilaginosa	9.3 [b]	18.3 [a]	n.a	15.0 [a]	n.a	13.0 [b]	n.a	11.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Schizosaccharomyces octosporus	14.3 [a]	37.8 [a]	13.3 [a]	37.8 [a]	3.2 [c]	15.0 [a]	n.a	7.7 [c]	n.a	10.0 [b]	n.a	n.a	n.a	n.a	n.a	n.a	4.7 [c]	6.7 [c]

Key to Table: The results are obtained by the agar disc diffusion method. The data were means of at least three replicate tests and are expressed as the area (mm in diameter) in which microbial growth was either completely inhibited or slowed down. [a] Microbial growth completely inhibited; [b] growth slowed down markedly; [c] growth somewhat affected; n.a. no activity.

On the other hand, the activity spectrum of pepper berry and leaves were relatively narrow. In particular, with the exception of the hexane extracts, pepper berry and leaves showed no activity against the three lactic acid bacteria tested, namely *Lactobacillus plantarum, Oenococcus oeni and Pediococcus cerevisiae*. This is interesting as although lactic acid bacteria can cause spoilage in some products, they are beneficial microorganisms in a number of other foods including cheese, yoghurt, and many fermented meat and vegetable products such as salami and sauerkraut. This phenomenon was also observed in several other native plant extracts. This, coupled with the observation that many of the native plant extracts exhibited inhibitory activity against several common foodborne human pathogens, may prove very useful for these products. The selective antimicrobial activities of these extracts mean that, when used in these products, they can inhibit the pathogenic and spoilage bacteria while not affecting the normal fermentation process of lactic acid bacteria.

Of the three types of extracts (methanol, water and hexane), methanol extracts, in general, showed the strongest activity, while the activities of water and hexane extracts were comparable in most cases. It should be noted, however, that the differences in the activities against spoilage bacteria between the three types of extracts were much less compared with their differences in the activities against foodborne pathogens. Furthermore, hexane extracts showed a broader spectrum of activity than both methanol and water extracts.

3.1.3. Activity against common food spoilage yeasts and moulds

The activities of Australian native plant extracts against nine of the common food spoilage yeasts are presented in Tables 3-5. Of the 19 bushfoods tested, 10 showed various degrees of inhibitory activity against the yeasts. Again, the strongest activities were shown by extracts of native pepper berry and leaves, especially against the yeast *Schizosaccharomyces octosporus*. These were followed by eucalyptus oil, forest berry, lemon myrtle, bush tomatoes and lemon ironbark, while the activities of the remaining plant extracts were relatively insignificant.

Pepper berry and leaves, together with lemon myrtle and eucalyptus oil, also showed the broadest spectrum of activity, exhibiting an inhibitory effect against all the nine yeast species tested. Forest berry also showed a broad spectrum of activity, exhibiting an inhibiting effect against seven of the nine yeasts.

Of the three solvents used, hexane appeared to be the most efficient in extracting materials with inhibitory activity against yeasts. This was followed by methanol while water was the least efficient. This is in contrast to the results obtained for activities against pathogenic and spoilage bacteria where methanol extracts showed the strongest and broadest activities.

These extracts were also tested for antifungal activities against several common food spoilage moulds including *Monilia fructicola* and *Botrytis cinerea*. However, none of the extracts showed a significant activity against the moulds tested.

3.2. Antioxidant properties of Australian bushfoods

3.2.1. General antioxidant activity in Australian bushfoods

Methanol extraction was carried out on 17 native plant foods (not acacia and eucalyptus oil). Antioxidant activity of the extracts was tested using two methods: β-carotene bleaching and the DPPH method. The former method measures the general antioxidant activity while the latter determines the free radical scavenging ability of the extracts. In addition, total phenolic contents of the extracts were also determined. The results of the tests are presented in Table 6.

All the bushfoods tested were found to possess some antioxidant activities, however the activities varied considerably. The activity, as determined by the β-carotene bleaching method, was highest in forest berry, followed by lemon aspen, Davidson plum and mountain pepper. Bunya nuts, on the other hand, had the lowest activity.

The native plant products also exhibited free radical scavenging activities, however these also varied considerably among the extracts. Native mint was found to have the greatest free radical scavenging activity, followed by lemon ironbark, quandong, forest berry, lemon myrtle and aniseed myrtle. Davidson plum, lemon aspen, wild lime, riberry and Warrigal greens, on the other hand, indicated low free radical scavenging activities.

The total phenolic contents of the native plant extracts ranged from 18.4 to 98.1 mg GAE /L. The total phenolic content correlated better with the free radical scavenging activity than the general antioxidant activity (β-carotene bleaching ability).

3.2.2. Selection and preparation of native plants for in-depth analysis of antioxidant activity

Based on the initial results, five plants with relatively high antioxidant activities were selected for further analysis. These were *Eucalyptus olida* (forest berry), *Mentha australi*s (native mint), *Tasmannia lanceolata* (mountain pepper), *Backhousia citriodora* (lemon myrtle) and *Eucalyptus stragiana* (lemon ironbark). The forest berry samples were collected from two locations: one from East Gipsland, Vic. (*Eucalyptus olida* GL) and the other from the Northern Rivers, New South Wales (*Eucalyptus olida* NR). Only leaves of the plants were used in this study. The plant leaves were picked on farm or supplied by growers. Samples were immediately packed with dry ice and transported to the laboratory. The leaves were extracted sequentially with hexane, ethyl acetate and methanol to give three crude extracts each. The crude extracts were partitioned into six fractions: hexane - methanol soluble, hexane - hexane soluble, ethyl acetate - ethyl acetate soluble, ethyl acetate - water soluble, methanol - hexane soluble, and methanol - methanol soluble. The crude extracts and the fractions were analysed for antioxidant activity using the TEAC and QEAC methods described above. The two methods gave similar data trends and only the TEAC data are presented in this report (Figures 2-6).

Table 6. Antioxidant Activities in Australian Native Plant Products

Plant	Antioxidant activity[a] (%)	Free radical scavenging activity[b] (%)	Total Phenolic Content[c] (mg of GAE /L)
Cut Leaf Mintbush	67.0	12.4	18.4
Bunya Nuts	18.9	20.6	19.8
Warrigal Greens	53.7	7.1	22.4
Davidson Plum	86.0	1.2	22.5
Lemon Aspen	87.0	3.6	28.2
Illawara Plum	59.0	14.8	34.5
Aniseed Myrtle	40.6	55.6	45.7
Bush Tomato	62.7	20.7	59.9
Lemon Ironbark	51.2	59.5	60.3
Riberry	50.5	5.9	63.5
Wild Limes Small	52.4	4.5	67.9
Mountain Pepper	83.2	43.1	69.5
Pepper berry	66.3	-	70.3
Quandong	55.5	59.5	86.2
Lemon Myrtle	46.7	56.6	88.1
Forest berry	87.7	58.4	95.9
Native Mint	41.8	66.8	98.1

Keys to the table.

1. The data were means of at least three replicate tests.
2. [a] Antioxidant activity was determined using the β-carotene bleaching method and expressed as % of inhibition of bleaching.
3. [b] Free radical scavenging activity was determined using the DPPH method and expressed as % DPPH (measured as absorbance at 515 nm) reduced.
4. [c] Total phenolic content was determined using the Folin-Ciocalteu procedure and expressed as milligram per litre gallic acid equivalent (GAE).

3.2.3. Recovery of solid matter

Figure 2 shows the recovery of solid matter from the native plants in methanol, ethyl acetate and hexane extracts. For all the six different plants, methanol extracts contained the most solids by far, usually more than the combined amounts in the ethyl acetate and hexane extracts. Hexane extraction recovered more solids than ethyl acetate in four of the plants, namely *Tasmannia lanceolata* GL, *Eucalyptus olida* GL, *Backhousia citriodora* and *Eucalyptus olida* NR. Recoveries of solids were similar between hexane and ethyl acetate extracts for the remaining plants.

3.2.4. Antioxidant activity in crude extracts of native plants

Figure 3 shows the antioxidant activity in the three different extracts as measured by the TEAC method, where the antioxidant activity was expressed as μg TROLOX equivalent per g of plant dry matter.

Of the six native plants (including two *Eucalyptus olida* samples collected at different locations), *Eucalyptus stragiana* NR was found to have the highest antioxidant activity, followed by *Backhousia citriodora* and *E. olida* NR, which had similar activities, while *Tasmannia lanceolata* had the lowest activity.

Of the three solvents used (methanol, hexane and ethyl acetate), methanol was the most efficient in extracting antioxidant compounds, followed by ethyl acetate while no activity was found in the hexane extracts. This is most likely due to the low solubility of phenolic compounds in hexane which is an apolar solvent.

3.2.5. Antioxidant activity in different solvent fractions

The crude extracts of native plants were further fractionated using the solvent partition technique. In total, six fractions were produced for each plant and Figure 4 shows the antioxidant activity in the different fractions.

Of the six different fractions, the methanol soluble fraction of the methanol extracts contained the highest antioxidant activity. Relatively high antioxidant activity was also found in the water fraction of ethyl acetate extracts of *Backhousia citriodora*, *Mentha australis* GL, *Eucalyptus olida* NR and *Eucalyptus olida* GL. Antioxidant activities in the other fractions were either relatively minor or not observed.

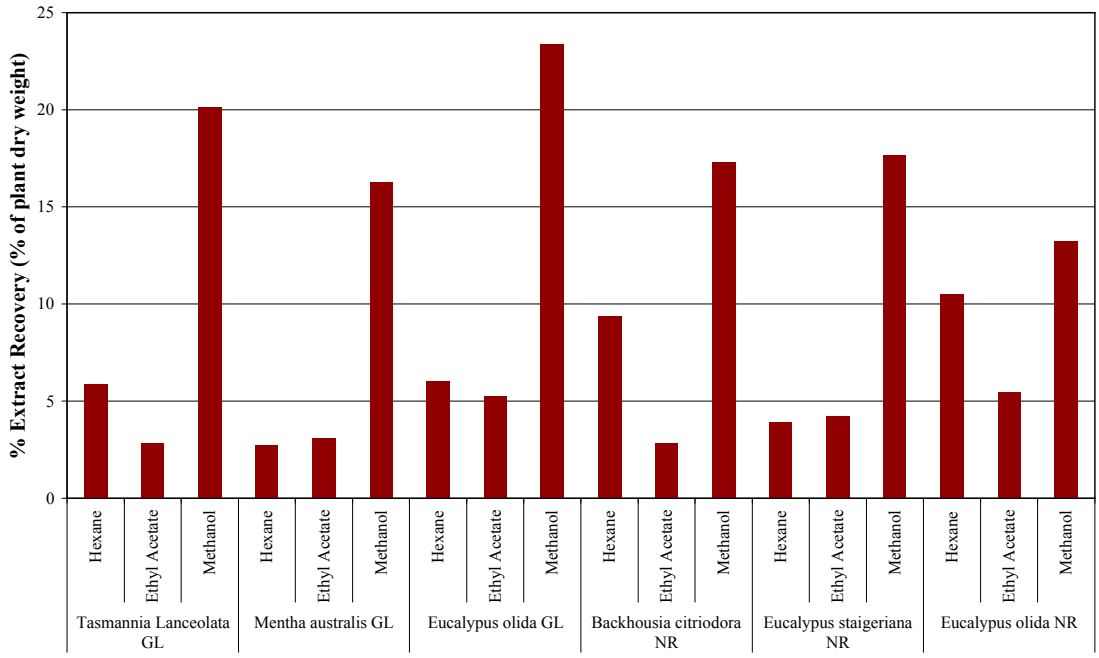


Figure 2. Recovery of solid matter from Australia native plant products in hexane, ethyl acetate and methanol extracts.

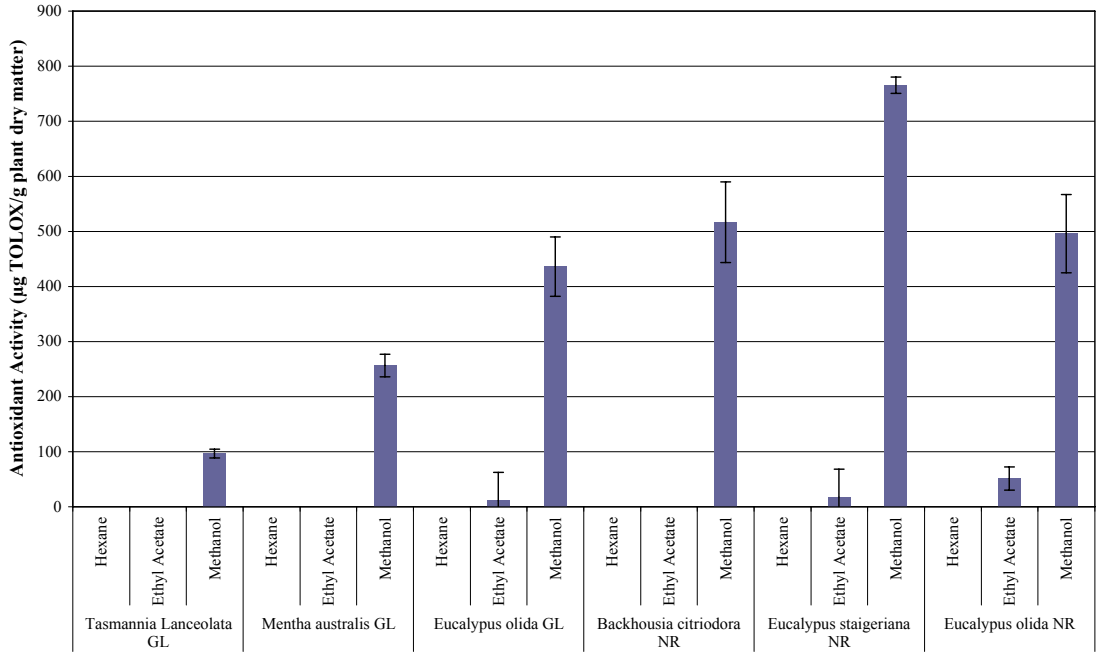


Figure 3. Antioxidant activity of Australia native plant products in hexane, ethyl acetate and methanol extracts

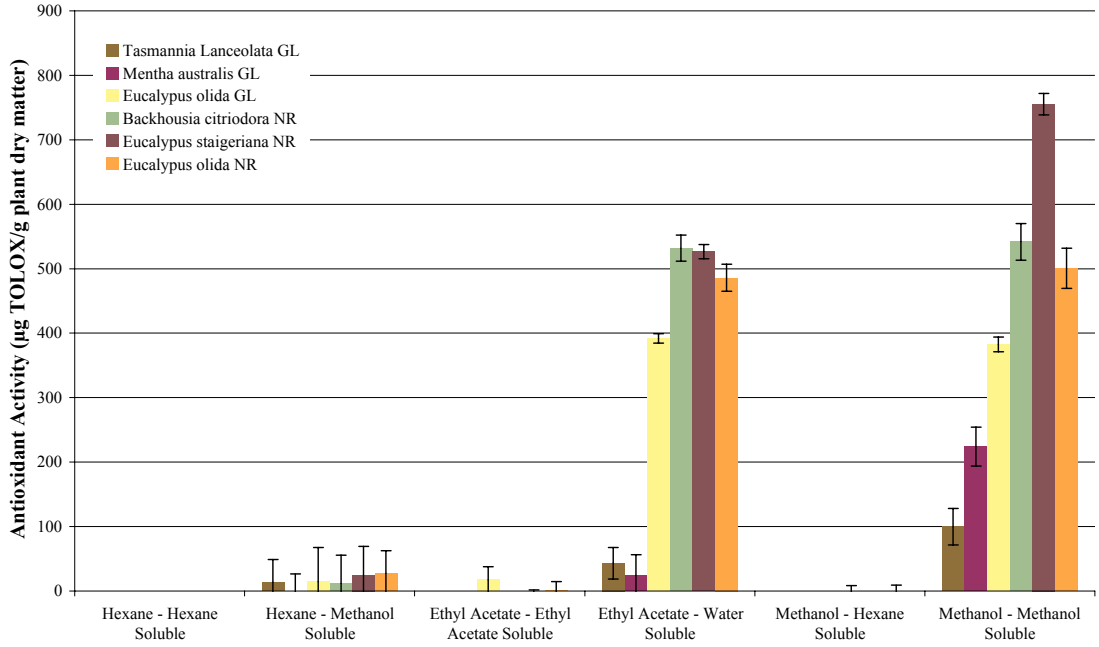


Figure 4. Antioxidant activity of Australian native plant products in different solvent fractions.

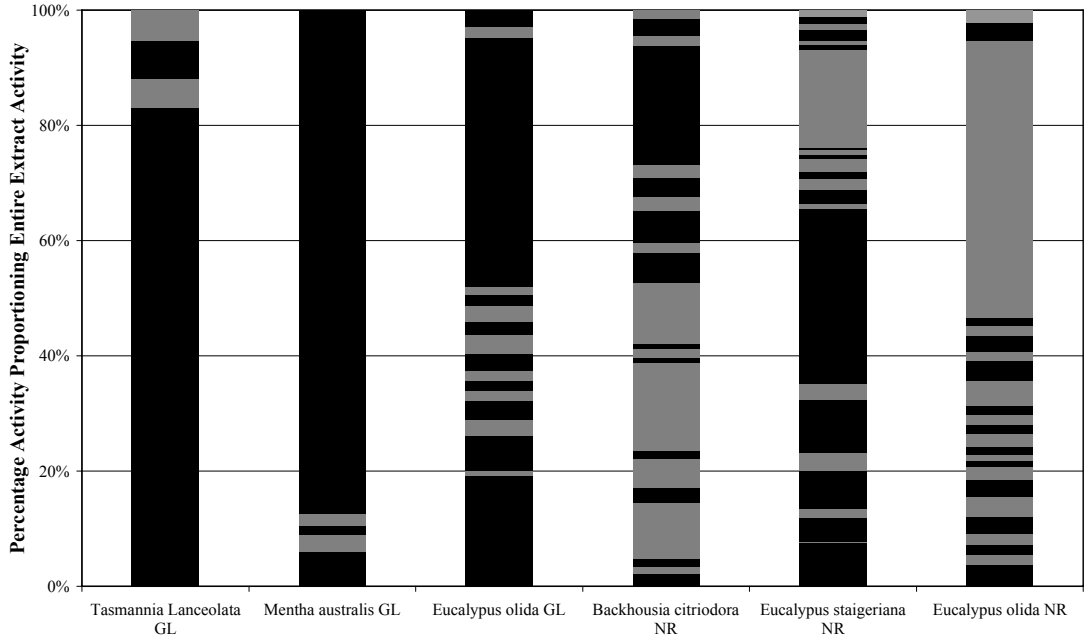


Figure 5. Number of HPLC peaks with antioxidant activity and their relative proportions in the total antioxidant activity of a particular fraction. Each bar represents an individual peak (compound) and the height of the bar represents the proportion of activity relative to the total activity of the fraction.

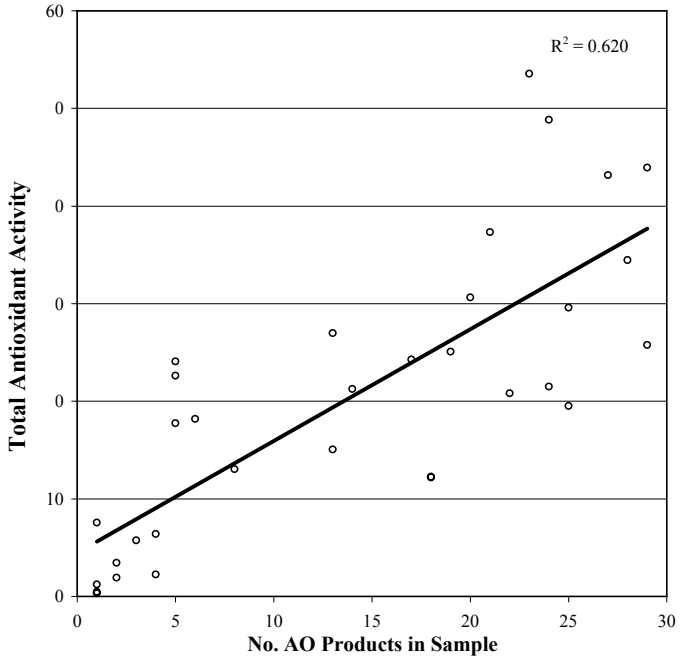


Figure 6. Correlation between the number of antioxidant compounds and the total antioxidant activity in the Australian native plant products

3.2.6. Analysis of native plant extracts by HPLC

The crude extracts and their six different fractions from the studied native plants were further analysed by reversed-phase HPLC to determine the character of the active compounds. The HPLC system was equipped with a post-column reagent mixing system that allows the TEAC reagents to be added to the HPLC eluent post-column, thus enabling the peaks with antioxidant activity to be identified online. Figure 5 shows the number of HPLC peaks (or compound if the peak is well resolved) with antioxidant activity and their relative proportions in the total antioxidant activity of different solvent fractions.

The number of compounds with antioxidant activity varied markedly in different plants and in the different solvent fractions. For example, only six antioxidant compounds were found in the various factions of *T. lanceolata* GL, whereas 47 were found in those of *Eucalyptus stragiana* NR. For the ethyl acetate extract of *Eucalyptus stragiana*, the water fraction contained 29 antioxidant compounds while the ethyl acetate fraction was found to contain 18 active compounds.

Figures 6 shows the correlation between the number of antioxidant compounds and the total antioxidant activity found in the native plant products. As can be seen, these two measurements were positively correlated, which means that the higher the number of antioxidant compounds found in a plant, the greater the total antioxidant activity in that plant. This implies that there was not one or a few compounds with exceptionally high antioxidant activity that accounts for the majority of the total activity observed. Rather, the total activity was well spread in various different compounds.

Figures 7-11 show the typical examples of HPLC profiles of antioxidant compounds in native plant extracts and their various solvent fractions. Each crude extract and each solvent faction was analysed by HPLC, but for brevity of the report, those HPLC profiles are not shown. Each HPLC chromatogram had two absorbance recordings. The bottom profile is the normal recording of the HPLC photodiode array detector, while the top profile is the recording of a UV detector after the injection of TEAC reagents post-column, thus indicating the peaks with active antioxidants. The bottom charts of each figure showed the UV absorption profile of each of the identified active compounds over an UV spectrum of 240-400 nm. Each profile was characteristic of a particular compound. However, the information alone is not sufficient to give positive identification of the compound. For such identification, analysis of the peaks by HPLC-MS is required.

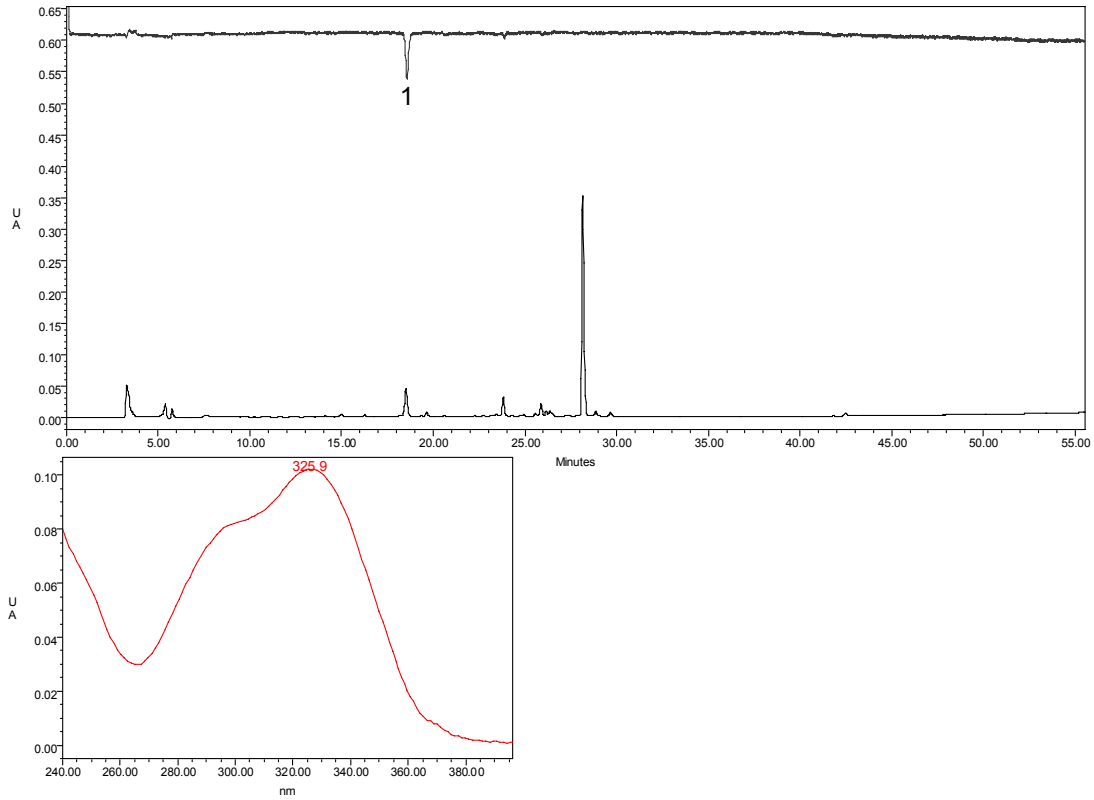


Figure 7. HPLC profile of the water fraction of the ethyl acetate extract of *T. lanceolata*. Numbered peaks contained active antioxidant compounds; the bottom chart shows the UV absorption profile of the numbered active peaks.

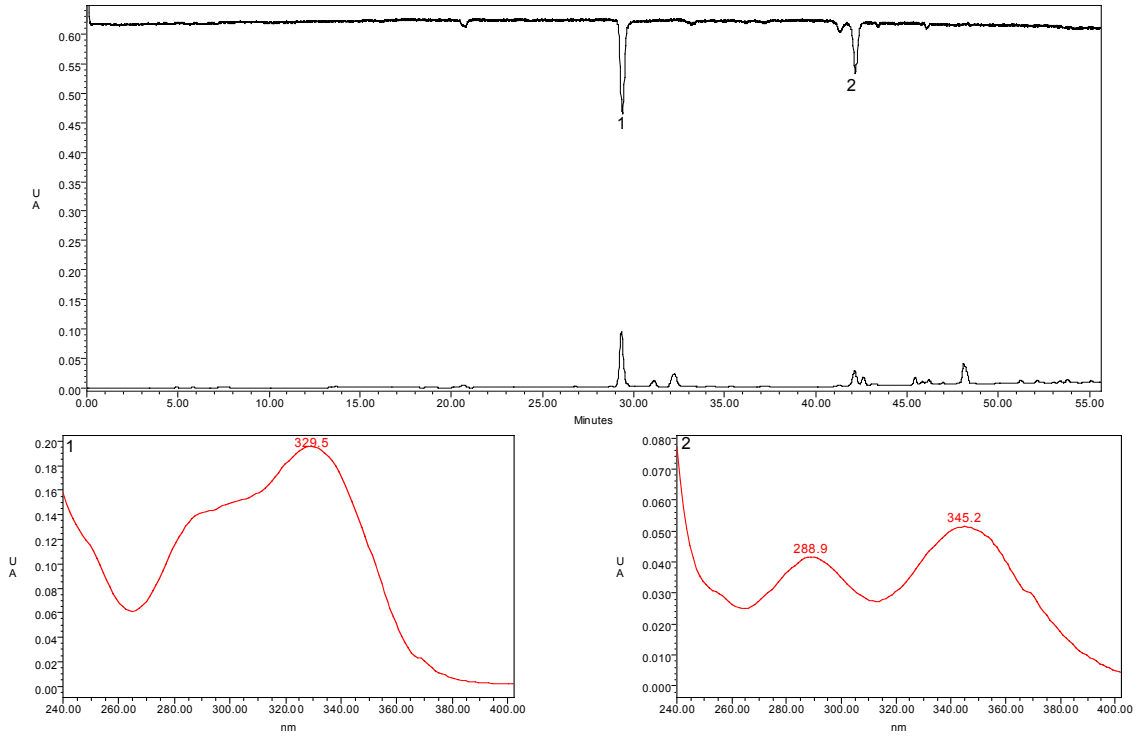


Figure 8. HPLC profile of the ethyl estate fraction of the ethyl acetate extract of *M australis*. Numbered peaks contained active antioxidant compounds; the bottom chart shows the UV absorption profile of the numbered active peaks.

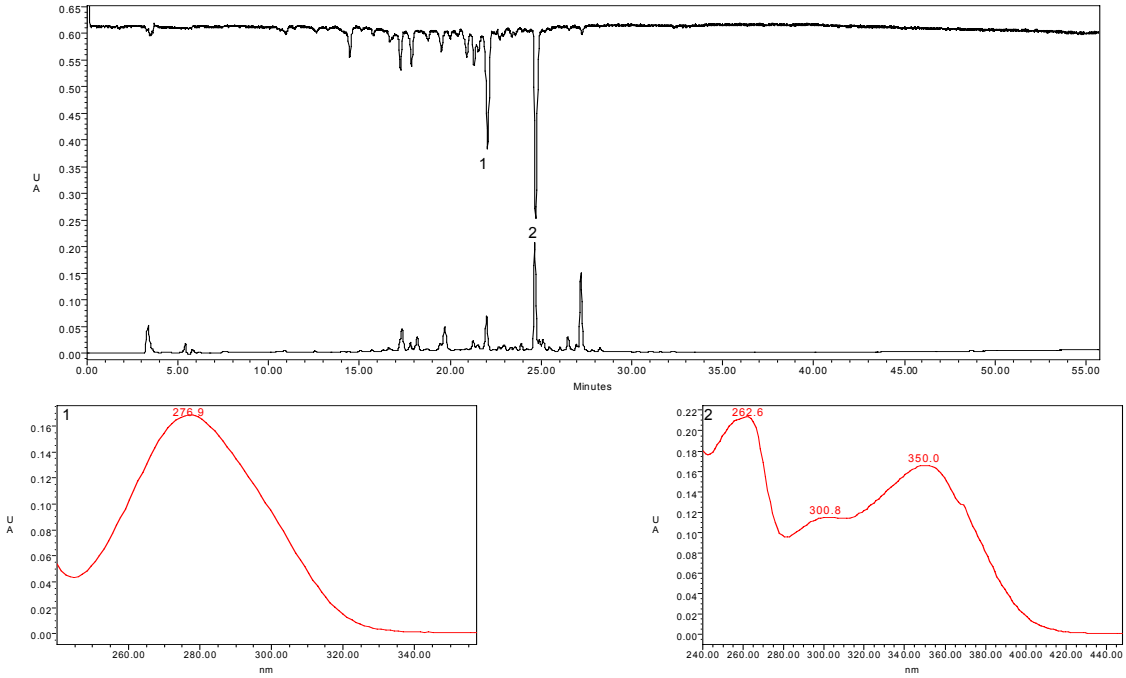


Figure 9. HPLC profile of the water fraction of the ethyl acetate extract of *E. olida*. Numbered peaks contained active antioxidant compounds; the bottom chart shows the UV absorption profile of the numbered active peaks.

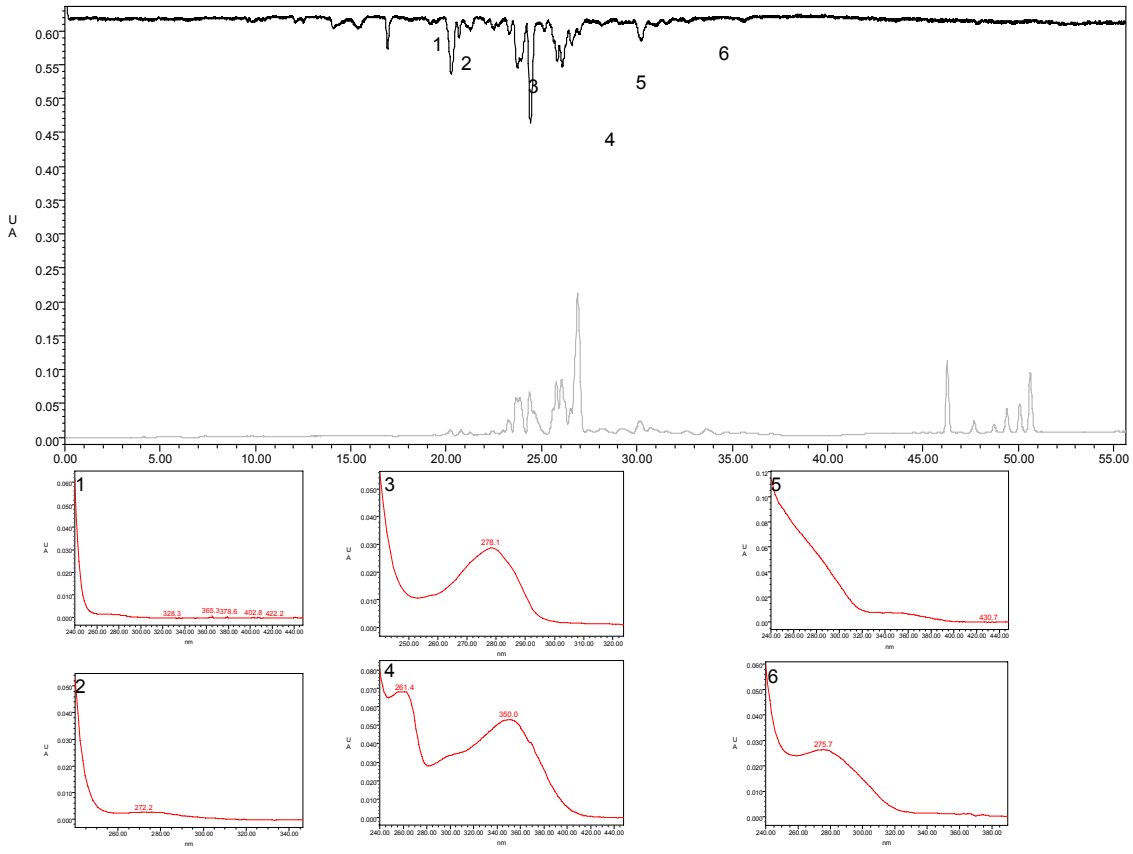


Figure 10. HPLC profile of the crude ethyl acetate extract of *B. citriodora*. Numbered peaks contained active antioxidant compounds; the bottom chart shows the UV absorption profile of the numbered active peaks.

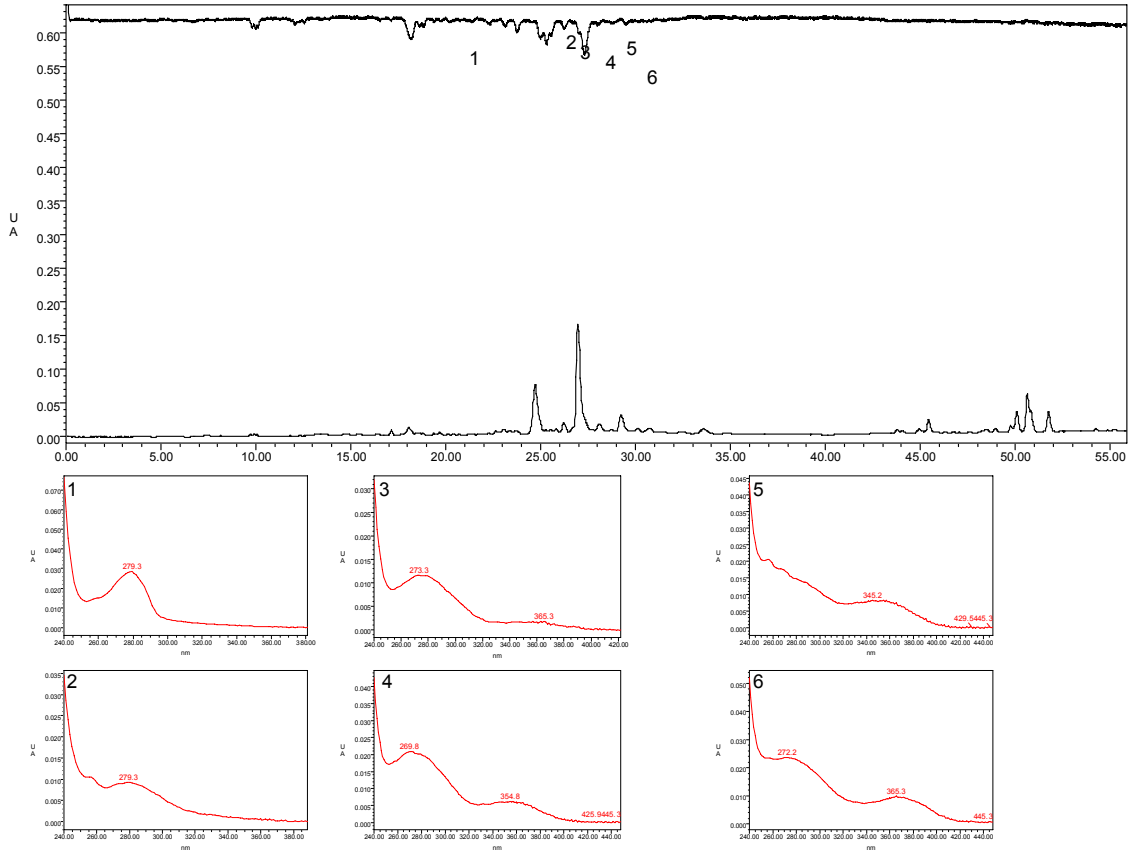


Figure 11. HPLC profile of the crude ethyl acetate extract of *E. stragiana* NR. Numbered peaks contained active antioxidant compounds; the bottom chart shows the UV absorption profile of the numbered active peaks.

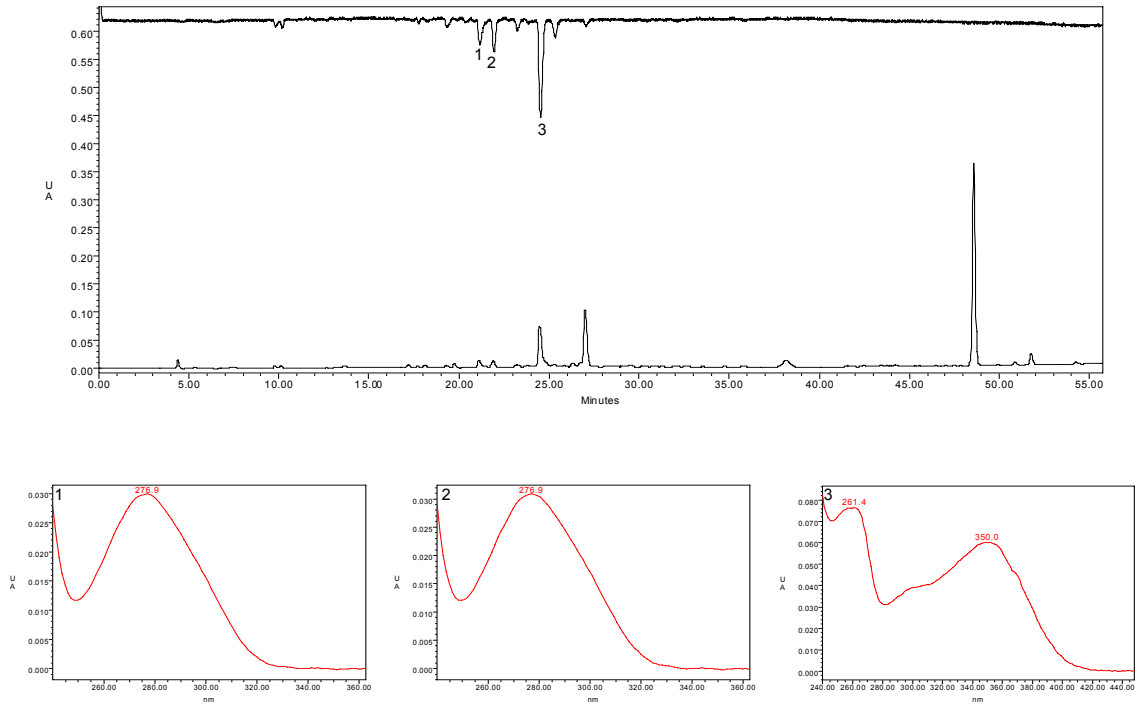


Figure 12. HPLC profile of the crude ethyl acetate extract of *E. stragiana* GL Numbered peaks contained active antioxidant compounds; the bottom chart shows the UV absorption profile of the numbered active peaks.

3.3. Emulsifying properties of wattle seed extracts

3.3.1. Extraction and characterisation of proteins

The proximate analysis of both whole wattle seed and its dehulled (inside) portion was measured (Table 7). Whole wattle seed was higher in fibre and total carbohydrates than the inside portion. However, the inside portion was significantly higher (almost double) in protein, fat and water-soluble carbohydrates. The result for the whole seeds was in agreement with Brand et al. (1985) who studied the composition of various Australian native plants including several from the *Acacia* family. Our results, however, also show that the hard outer coat made significant contributions to the mineral (ash) and fibre contents of the plant.

Table 7. Proximate analysis of wattle seed*

Component	Whole seed (%)	Inside portion (%)
Water	2.13	2.54
Protein (g N x 6.5)	18.20	34.45
Fat	5.40	10.31
Neutral Detergent Fibre	32.10	16.76
Ash	3.70	2.80
Carbohydrates (by difference)	38.47	33.14
Water Soluble Carbohydrates	5.81	11.03

*Data are means of three replicates.

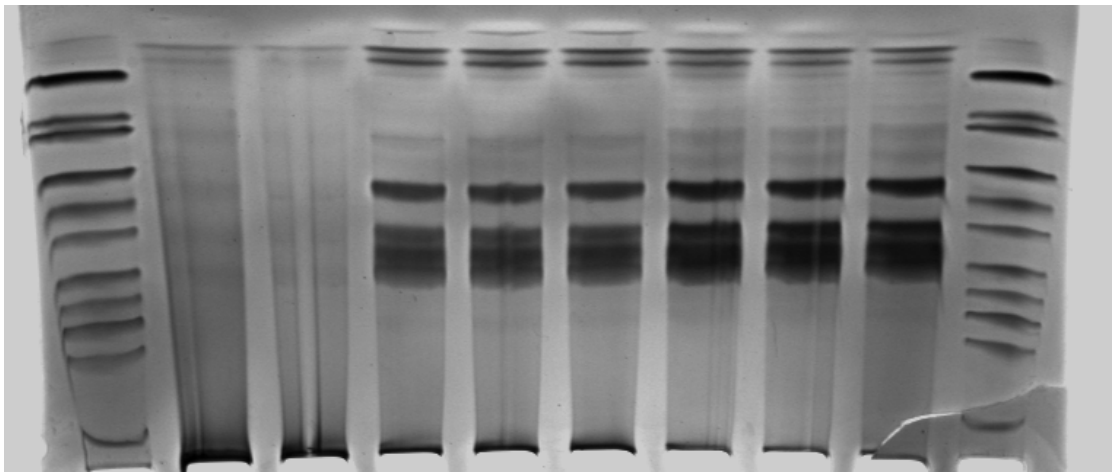
Table 8. Recovery of wattle seed extracts*

Sample	Extract	Yield (g/100g sample)	Protein content (%)	Fibre content (wt %)	Water soluble carbohydrates (wt %)
Whole seed	Water	34.49	26.00	< 0.10	18.50
	Alkali	11.33	52.65	<0.10	ND
	Ethanol	1.79	13.00	<0.10	10.71
	Residue	49.74	7.15	59.58	ND
Inside portion	Water	67.77	44.85	<0.10	17.90
	Alkali	2.02	41.60	<0.10	ND
	Ethanol	1.29	5.85	<0.10	9.89
	Residue	22.59	2.6	63.93	ND

*Data are means of three replicates.

ND, not determined.

Results of material yield after extraction (Table 8) also show a much higher fibre level, suggesting that most of insoluble materials from the whole seeds were fibre. Incidentally, the residue obtained after extraction of the inside portion, although much smaller in total material, had much higher fibre content than that obtained from extracting the whole seed. This would suggest that it was much easier to extract the soluble components from the inside portion than from the whole seed. Table 8 also shows, conclusively, that there were more water-soluble components in both sets of samples than the other constituents extracted, especially in the inside portion whereby water-soluble extract yielded 67.77g solids/100g sample compared with 34.49g solids/100g sample in the whole seed. It also appeared that alkali and ethanol were better able to extract dry matter from the whole seed than from the inside portion. Furthermore, these alkali- and ethanol-soluble extracts contained significantly more proteins. The results would indicate the nature and properties of constituents wattle seed, with the extracts being composed mainly of proteins and water-soluble carbohydrates.



1 2 3 4 5 6 7 8 9 10

Figure 13. Molecular weight analysis by SDS-PAGE of protein extracts from wattle seed. Lanes 1 & 10: Sigma markers from 14.2 kDa to 205 kDa; lanes 2 & 3: ethanol-soluble extracts from whole seed and inside portion respectively; lanes 4 & 5: alkali-soluble extracts from whole seed and inside portion respectively; lanes 6 & 7: water-soluble extracts from whole seed and inside portion respectively; lanes 8 & 9: wattle seed powders from whole seed and inside portion respectively.

Results of SDS-PAGE analysis of the extracts from whole wattle seed are shown in Figure 13. It is in agreement with the % protein data in Table 8 in that ethanol extracts (lane 2 & 3) had very faint bands, indicating much lower protein content compared to alkali (lanes 4 & 5) and water extracts (lanes 6 & 7). The total number of bands as indicated in the whole seed lanes (8 & 9) was 12, which is slightly less than the 14 bands commonly reported for soybean protein after SDS-PAGE analysis (deMan, 1999). Results also indicate that most of the protein bands found in the whole seeds are also in the inside portion, the only difference being that the outer shell appears to only reduce the intensity of the bands. This relative intensity between bands of whole seed extracts and those from the inside portion persists in most samples (also in agreement with the % protein data as shown in Table 8). Interestingly, most of the bands found in the alkali extracts were also present in the water extracts. Although the ethanol extracts were not well defined, most likely due to their low protein concentrations, they probably have similar molecular weight profile to their water and alkali counterparts, ranging in size from about 13.3 kDa to 90.7 kDa. In both water and alkali extracts, the major bands were in the 27.6 kDa to 60.9 kDa range. These results suggest that the alkali-soluble extracts were possibly conjugated multi units of the water extracts which were reduced to similar sizes due to experimental conditions employed for the SDS-PAGE analysis, e.g., reduction in disulphide bonds.

Results of CE analysis appear to confirm the data obtained for other protein analyses. All extracts appeared to have similar retention times of around 4 min. The proteins appear as one major peak under CE analysis which is typical of seed proteins (Basha, 1997). There were also some minor peaks but these were not properly defined in this experiment probably because the experiments were performed under non-denaturing conditions. Rice storage proteins from rice were shown by Agboola et al. (2005) to have several major peaks when the CE was carried out under denaturing conditions.

3.3.2. Emulsifying properties of water extracts from whole wattle seed

The emulsifying properties of the wattle seed has been performed using water extracts because emulsifiers were expected to be soluble in the continuous phase of emulsions, in this case, oil-in-water emulsion system. Emulsions containing 20% or 50% oil were generally liquid, although 50% oil emulsions were more viscous. Comparatively, those emulsions containing 80% oil were gel-like in nature, oiling off within 24 hrs irrespective of storage temperature, salt level or pH.

As shown in Table 9, the viscosity of the extract was highly dependent on the pH, a situation which, in turn, affected the ease of formation of the emulsions and their stability under the various conditions in this study. According to Walstra (1983), viscosity of the continuous phase adversely affects the ability of the oil particles to deform and break down into smaller globules, consequently, the large particle so formed at high viscosity may destabilise faster. This pH effect could be due to either the protein (proximity to isoelectric point) or the carbohydrate components of the extracts.

Table 9. Viscosity (in mPa)* of wattle extracts and their emulsions at different pH values

pH	Extract (supernatant)	20 % oil-in-water emulsion	50 % oil-in-water emulsion
3	2.8	4.9	29.5
4	3.4	2.22	14.06
5	48.2	64.7	479.0
7	810.0	140.0	966.0
9	3.82	50.1	230.5

*Data are means of three replicates.

Emulsions were studied by measuring d_{43} particle size average and size distribution under the influence of pH, salt concentration and retorting. As shown in Figures 14 and 15A, 20 % oil-in-water emulsions made at pHs 3, 4, 5 and 9 were smaller is size (emulsion particles ranged between 0.1 and 10 μm) and consequently more stable compared to the emulsions formed at pH 7 (considerably higher extract viscosity and a significant group of particles ranged between 10 and 100 μm). The same trend was observed for 50% oil-in-water emulsions at pHs 3, 4 and 5, albeit in much higher average size ranges (Figures 15B, 15D and 15B). Significantly though, 50% emulsions at pH 7 had high particle size averages that declined for the first 3 days after which they slightly increased in size for the remaining 4 days of observation. This increase was, however, accompanied by the emulsion breaking and oiling off on the surface. Comparatively, 50% oil-in-water emulsions at pH 9 consistently increased in average size throughout most of the observation period (Figures 15B and 15D) and were not accompanied by any significant oiling off. The 50% oil-in-water emulsions were also relatively unstable enough for the storage conditions to have an effect especially at pHs 7 and 9. It would appear that the higher oil content and the increased interfacial area created without an accompanying increase in the amount of surfactant (protein level reduced from 1.1% for 20% oil emulsion to 0.43% for 50% oil emulsion) led to such a significant change in the stability of 50% oil-in-water emulsions. Protein-stabilised emulsions generally have lowered average particle size and good stability if the surface coverage is adequate (Agboola et al., 1998; Fang and Dalgleish, 1993). The results in this study would also suggests that water-soluble extract of wattle seed contains materials suitable for stabilising even high oil content emulsions (most probably the proteins).

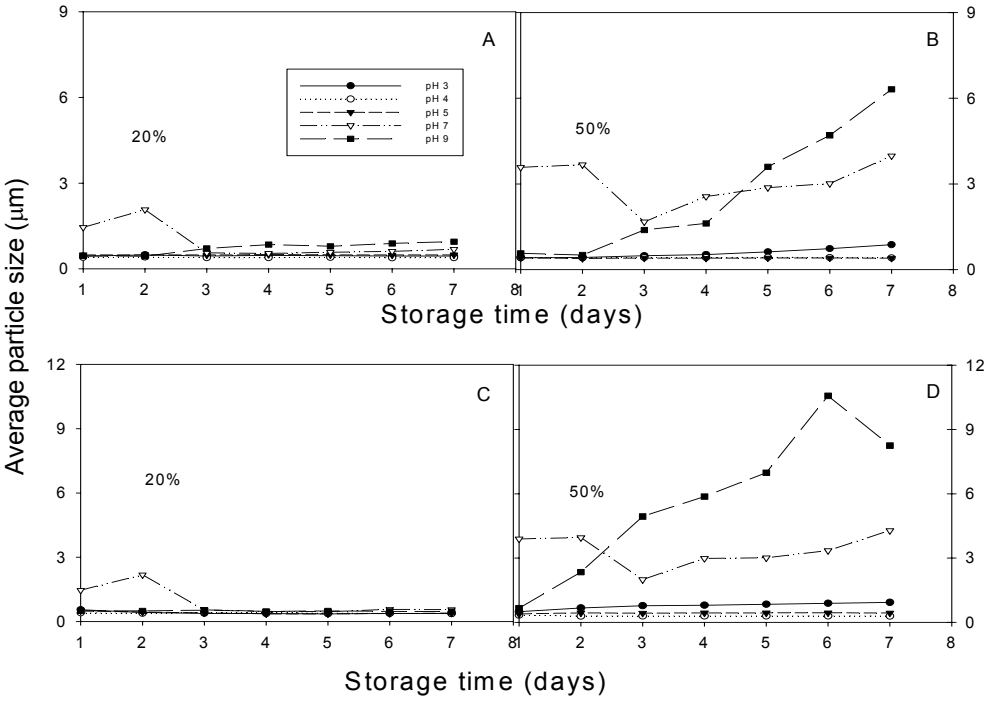


Figure 14. Effect of storage time on average particle size (d_{43}) of 20% or 50% oil-in-water emulsions formed with water extract from ground whole wattle seed. Emulsions were stored either at refrigeration temperature (A & B) or incubated at 25°C (C & D).

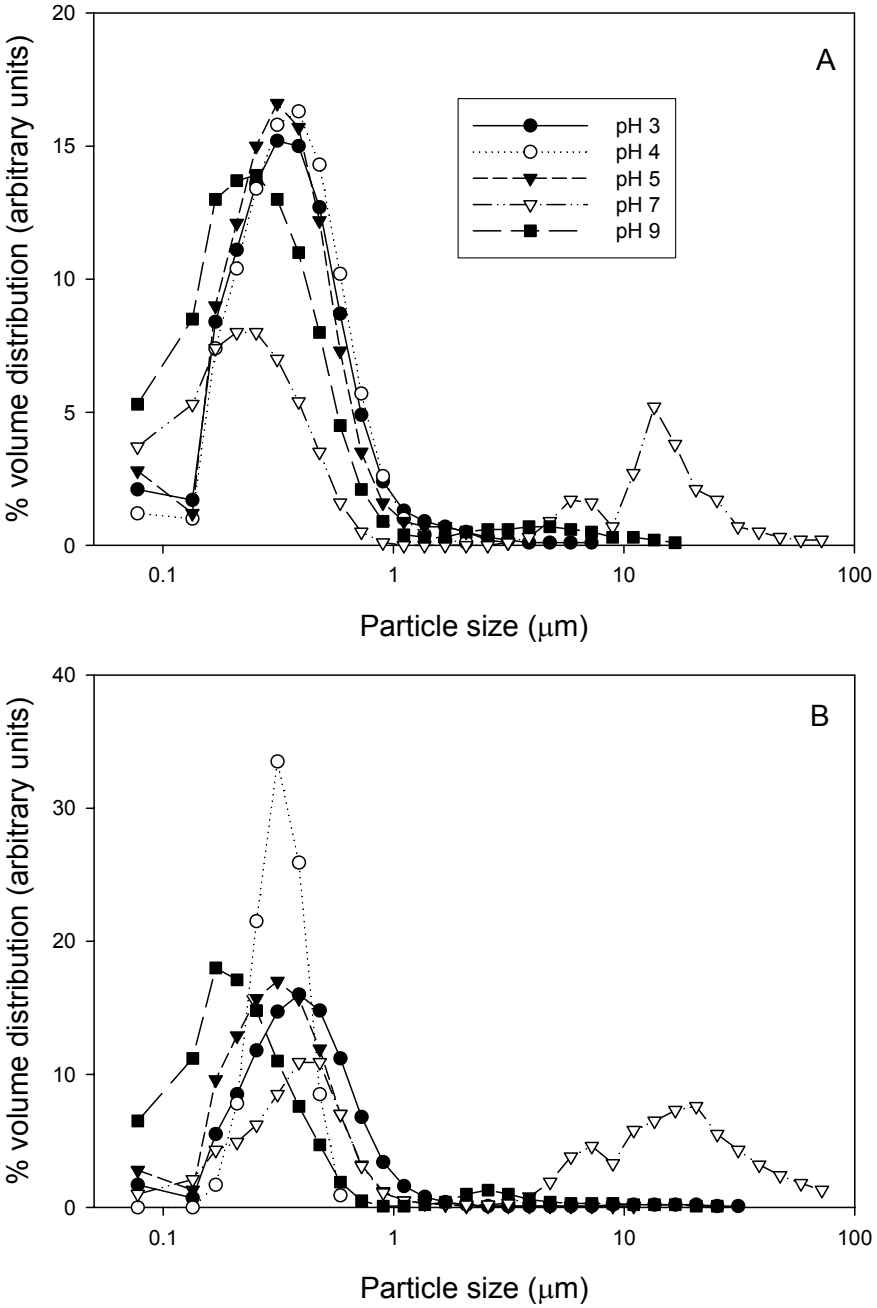


Figure 15. Particle size distribution of 20% (A) and (B) 50% oil-in-water emulsions formed with water extract from ground wattle seed at different pH values.

The influence of salt concentration and retorting on the stability of 20% oil-in-water emulsions formed at pHs 4 and 5 are shown in Figure 16. In general, emulsions formed at pH 4 formed smaller particles (lower d43 average size) and were more stable under storage than those at pH 5 probably due to increased viscosity of the extract at the higher pH as noted above (see Table 9). The influence of increasing NaCl concentration was not very significant at pH 4 (Figure 16A) probably due to the inherent stability of the emulsions but at pH 5, we clearly see a significantly less stable emulsion as the salt concentration increased to 0.5% and also at 1% (Figure 16B). Overall, though, the emulsions were still stable (no breaking and no oiling off) even though the size average increased. It would appear that salt screening of the electrical double layer of the protein-covered emulsion droplets reduced electrostatic repulsion between the droplets, leading to flocculation and probably coalescence of the droplets (Walstra, 1987). It is also possible that steric repulsion between the particles was reduced by the binding of cations to the wattle seed proteins on the droplet surfaces. Brooksbank *et al.* (1993) has shown that monovalent cations like Na^+ adsorbed to protein (β-casein) surfaces led to a reduction in the steric repulsion between latex particles.

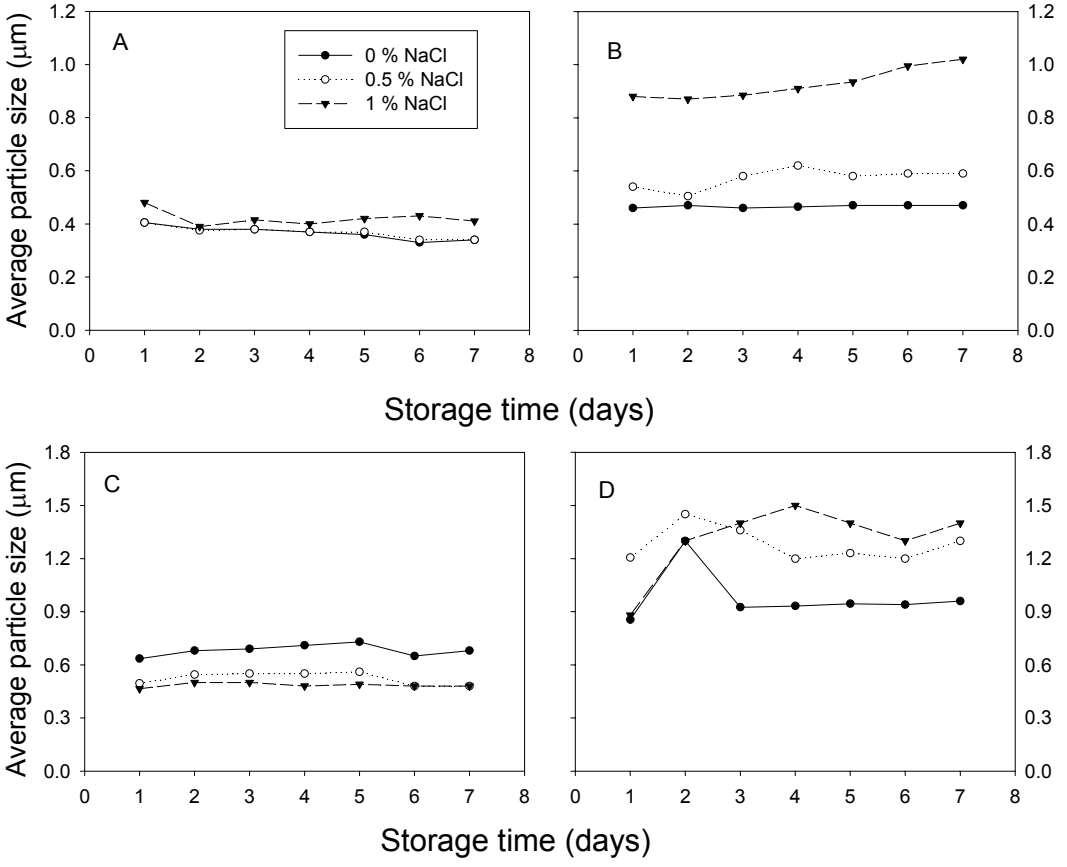


Figure 16. Influence of NaCl content and retorting (C&D) on the stability (d_{43} average size) of wattle seed extract-stabilized 20% oil-in-water emulsions formed at pH 4 (A, C) and pH 5 (B, D).

Retorting the emulsions caused a slight increase in the d_{43} average size of both sets of emulsions but the emulsions formed at pH 4 remained very stable throughout the observation period of seven days (Figure 16C). Conversely, emulsions at pH 5 were stable only within the first two days, forming a significant cream layer afterwards. It is significant to note that retorting did not cause immediate separation at either pH level and suggests that the wattle proteins are quite heat labile after adsorption onto the oil surface. It is also possible that the carbohydrates in the serum layer acted as stabilizers for the emulsion system.

Our study has shown that water soluble extracts from wattle seed contain predominantly proteins and carbohydrates, mainly within the cotyledons. These extracts have also been shown to be an excellent emulsifier/stabiliser for oil-in-water emulsion system even at relatively high oil content and very low protein levels. It is, however, important to understand the nature of these proteins, especially the possibility that some may be anti-nutritional factors and how processing for this could affect utilization of the extracts in the food industry. Research also continues on how to elucidate the relative roles of the two major components of wattle seed extracts, proteins and soluble carbohydrates, in the stabilisation of oil-in-water emulsions.

4. Conclusions

This study was undertaken with the aims of generating reliable data on the antimicrobial and antioxidant properties of bushfoods, expanding the application of bushfoods to areas including antimicrobials, antioxidants and emulsifiers for food applications and developing antimicrobial, antioxidant and emulsifying agents for use as natural substitutes for their synthetic counterparts.

With the results obtained in the study, the following conclusions can be drawn:

- Many native plant food products possess antimicrobial activities and several have relatively strong activities against a number of important foodborne human pathogens, food spoilage bacteria and yeasts. Mountain pepper berry and leaves (*Tasmannia lanceolata*) were found to have the strongest antimicrobial activity, while eucalyptus oil is effective against the broadest spectrum of pathogens. All the other native plants, with the exception of Warrigal greens, also have some degree of activities against some of the microorganisms, by either inhibiting or slowing down their growth. Thus, the findings of this study are able confirm the anecdotal evidence held by the industry in this respect, and the information can be used by the bushfood industry to promote their products.

- Interestingly, some extracts, such as pepper berry and leaves, have strong activities against human pathogens and spoilage bacteria but have no activity against lactic acid bacteria (LAB). This information may prove very useful as lactic acid bacteria are beneficial microorganisms in a number of food products including cheese, yoghurt, and many fermented meat and vegetable products such as salami and sauerkraut. The selective antimicrobial activities of these extracts mean that, when used in these products, they can inhibit the pathogenic and spoilage bacteria while not affecting the normal fermentation process of lactic acid bacteria.

- The native plant food products are, however, ineffective against all the spoilage moulds studied.

- All the native plants were found to possess some antioxidant activities, however the activities varied considerably between plants. The activity, as determined by the β-carotene bleaching method, was highest in forest berry, followed by lemon aspen, Davidson plum and mountain pepper. The native plant extracts also possessed free radical scavenging activities, which also varied markedly among the plants. Native mint was found to have the greatest free radical scavenging activity, followed by lemon ironbark, quandong, forest berry, lemon myrtle and aniseed myrtle.

- Most of the antioxidant activities were found in the methanol extract while little activity was found in the hexane extract. This suggests that the antioxidants are likely to be polar compounds.

- Wattle seed extracts have strong emulsifying powers. Even at very low protein concentrations (0.17-1.12%), stable oil-in-water emulsions can be formed with lasting stability. For the purpose of comparison, milk, which is an emulsion stabilised primarily by milk protein, contains more than 3% of protein.

- The stability of the emulsions was affected by pH, with the emulsions more stable at acidic than at neutral or alkaline pH values. It's worthwhile to note that many emulsion type food products, such as mayonnaise and salad dressings, are acidic, and wattle seed extracts can be used in these products. The emulsions are very heat stable and can withstand processing at temperatures as high as 115°C for 30 minutes.

- Overall, wattle seed extracts appear to have a great potential as emulsifiers and stabilisers for the food industry, especially at low pH levels.

5. References

Adam, M.R. & Moss, M.O. (2000) *Food microbiology*, 2nd edn., Royal Society of Chemistry, Cambridge, UK.

Agboola, S., Ng, D. & Mills, D. (2005) Characterisation and functional properties of Australian rice protein isolates. *J. Cereal Sci*. *41*, 283-290.

Agboola, S.O., Singh, H., Munro, P.A., Dalgleish, D.G. & Singh, A.M. (1998) Destabilization of oil-in-water emulsions formed using highly hydrolyzed whey proteins. *J. Agric. Food Chem*. *46*, 84-90.

AOAC. (1996) Official Methods of Analysis, 16th edn., Association of Official Analytical Chemists: Washington DC.

Basha, S.M. (1997) Separation of peanut proteins by capillary electrophoresis. *J. Agric. Food Chem*. *45*, 400-402.

Bates, J. R. & Bodnaruk, P.W. (2003) *Clostridium perfringens*. In: *Foodborne Microorganisms of Public Health Significance*. Hockings, A.D. ed. AIFST NSW Branch Food Microbiology Group: Sydney, Australia.

Brand, J.C., Cherikoff, V. & Truswell, A.S. (1985) The nutritional composition of Australian Aboriginal bushfoods 3. Seeds and nuts. *Food Technol. Australia*. *37*, 275-278.

Branen, A.L. (1993) Introduction to use of antimicrobials. In: *Antimicrobials in foods*, 2nd edn. Davidson P.M. & Brannen A.L. eds. Marcel Dekker: NY.

Brooksbank, D.V., Davidson, C.M., Horne, D.S. & Leaver, J. (1993) Influence of electrostatic interactions on b-casein layers adsorbed on polystyrene lattices. *J. Chem Soc. Faraday Trans*. *89*, 3419-3425.

Cherikoff, V. (2001) Personal communication.

Deak, T. & Beuchat, L.R. (1996) *Handbook of food spoilage yeasts*. CRC Press: Boca Raton, Florida.

deMan, J.M. (1999) *Principles of Food Chemistry*, 3rd edn., Aspen Publishers, Inc.: Maryland.

Fang, Y.& Dalgleish, D.G. (1993) Dimensions of adsorbed layers in oil-in-water emulsions stabilized by caseins. *J. Colloid Interface Sci*. *156*, 329-334.

Forbes-Smith, M. & Paton, J.E. (2002) Innovative products from Australian native foods; Report by Rural Industries Research and development Corporation (RIRDC) Publication No 02/109, Canberra, ACT, Australia.

Hasenhuettl, G.H. (1997) Overview of food emulsifiers. In: *Food emulsifiers and their applications*, Hasenhuettl G.H. & Hartel R.W. eds. Chapman & Hall: NY.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680-685.

McClements, D.J. (1999) *Food emulsions: principles, practice, and techniques*. CRC Press: Boca Raton, Florida.

Parish, M.E. & Dividson, P.M. (1993) Methods of evaluation. In: *Antimicrobials in foods*, 2nd edn., Davidson P.M. &.Brannen A.L. eds. Marcel Dekker: NY.

Peterson, D.M. (2001) Oats antioxidants. *J. Cereal Sc*. 20: 115-129.

Phelps, D.G. (1997) Feasibility of a sustainable bush food industry in Western Queensland. RIRDC Research Paper Series No. 97/37. Rural Industries Research and Development Corporation, Canberra, ACT.

Pitt, J.I. & Hocking, A.D. (1997) *Funji and food spoilage*. Chapman & Hall: London.

Stewart, C.M. (2003) *Staphylococcus aureus* and Staphylococcal enterotoxins. In: *Foodborne Microorganisms of Public Health Significance*. Hockings, A.D. ed. AIFST NSW Branch Food Microbiology Group: Sydney, Australia.

Walstra, P. (1983) Formation of emulsions. In *Encyclopedia of Emulsion Technology*, Becher, P. ed. Marcel Dekker: New York, New York, Vol. 1, pp 57-98.

Walstra, P. (1987) Physical principles of emulsion science. In *Food Structure and Behaviour*; Blanshard, J. & Lilliford, P.J., eds. Academic Press Ltd.: London, UK, pp 87-106.