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Food safety of three species of

NATIVE MINT

A report for the Rural Industries Research and Development Corporation

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Foreword

In recent times some members of the *Prostanthera* (the native mint bushes) genus have been used to a limited extent by bush food specialists in syrups, cordials, salsas, chutneys etc. One attractive feature of the mints is their attractive, clean, strongly minty eucalyptal fragrance.

The oils of two species have been reported to display anti-microbial activity. Mints of this genus should be investigated for commercial use. A mint which has good flavour and the prospect of acting as a preservative makes an excellent food ingredient if shown to be safe for human consumption.

This report develops methods for easy identification of the selected three species of *Prostanthera* and studies their characteristic texture, smell, taste and storage and cooking stability. It identifies the major chemical constituents of the oils, investigating the possible side effects after eating by studying published reports involving each constituent. It also looks into the anti-microbial activity of the oils from *Prostanthera.*

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Peter Core
Managing Director
Rural Industries Research and Development Corporation

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Executive Summary

The purpose of this project was to enhance the market potential for three *Prostanthera* species by screening each species for food safety and anti-microbial activities in the herb and in the manufactured product, thus gathering data to form a basis for food standards.

This broad aim was fulfilled by a number of studies which were meant to

- provide means and some reference criteria for the rapid identification of the three *Prostanthera* species as raw materials;
- identify the main chemicals present in each species and establish the toxicity status of each;
- clarify the toxicity to humans after ingestion of each specie;
- establish the stability of their primary marketable trait ---their fragrance;
- investigate their anti-microbial activity and the potential to serve as food preservative.

The first of these aims has been completed by the microscopic and macroscopic study coupled with the TLC results.

GCMS data identified most of the major lipophilic chemicals present at two time points, yielding thereby a lead to the possible toxicity of the herb oil and its stability to storage, to microwaving, and to baking. Three prominent components in the fresh oil of *Prostanthera rotundifolia* were detected but could not be identified.

From an extensive literature search centred on the chemicals defined by GCMS the conclusion was drawn that while ingestion of moderate quantities of the leaf was unlikely to produce untoward effects, conservatively a safe adult dose consists of about four and a half teaspoons of the ground dried leaf, or about 0.2 ml of the pure oil, assuming a safety limit that is parallel to that of Eucalypt oil.

Sensory tests pointed to a loss of fragrance with storage, the loss being only partial in the case of *Prostanthera incisa* and *Prostanthera rotundifolia* but quite complete in the case of *Prostanthera lasianthus*. The varying degree of loss was supported by GCMS data, which also indicated that the oil of *Prostanthera incisa* was more resistant to storage decay than that of *Prostanthera rotundifolia*.

The fragrance survived microwaving to a degree, but was completely destroyed by baking.

The intense bitterness of all three species of leaf was their most unattractive trait. This decreased slightly with storage but was even then still off-putting. However the oils were less bitter than expected. This lead to the speculation that the bitter taste may be due to other components as well as the terpenoids that were present in the oils.

The fresh oils from *Prostanthera incisa* and *Prostanthera rotundifolia* demonstrated activity against Gram positive bacteria, which activity was still evident in oils from herbs that had been stored for five months. That a number of terpenoid chemicals displayed anti-microbial behaviour had been reported by various groups. This and our data supported the conclusion that the anti-bacterial activity originated from the lipophilic components of the leaf. In relation to the inactivity of *Prostanthera lasianthus*, it is interesting to note that the levels of cineole present were found to be lower at both time intervals than those of the other two species, Table F.4.
When added to water, sugar solution, and oily solutions, the ground freshly dried leaves of all three species, with a single exception, showed temporary (one week duration) bacteriostatic action. This property should be further looked into if decisions are to be formed about its usefulness as a preservative.

Thus this study has fulfilled its original brief. In closing the report the following suggestions are put forward for consideration.

The compositions of the oils of *Prostanthera rotundifolia* and of *Prostanthera incisa* in this study were slightly different from that found by others (see Section F.2 a and F.2 b), hence giving rise to the suggestion that oil composition may be affected by environmental conditions, ie soil, climate, harvest period, period of sunlight, etc. The correlation between composition and other factors should be investigated since products with wider markets could be developed, for example specimens whose leaves retain the original fragrance but are devoid of bitterness, or whose oils show greater anti-microbial prowess.

The as yet un-identified chemicals in *Prostanthera rotundifolia* oil, as well as the flavonoid compounds in all three species, should be identified and included in an expanded safety investigation.

Bacteriostatic activity, which has been shown in this work to be present in *Prostanthera incisa* and *Prostanthera rotundifolia* merits deeper and more rigorous experimental work for commercial applications.

The intense bitterness of the *Prostanthera* leaves weighs against their inclusion in foods which are not highly sweetened. Developmental work before marketing should include ways of masking or removing altogether this taste while leaving the fragrance and anti-microbial property.

1. Introduction

The *Prostanthera* as a genus are native plants which are fairly common and readily grown. The members of this family possess a distinctive minty/ eucalytal fragrance. This, together with their dainty mauve coloured flowers render many *Prostanthera* species to be popular with many gardeners.

In recent years there has been increasing interest taken in the role of indigenous plants as raw materials. Native plants with various distinctive properties have been introduced into a number of diverse fields such as cosmetics, pharmaceuticals, toiletry, etc, including the important arena of cuisine. Due to their characteristic fragrance, a few *Prostanthera* species have been used by isolated chefs as an ingredient in the production of cordials, syrups, sauces and chutneys (personal communications from S. Hess- Buschmann, "Gold Coast Bush Foods" and from J. Robyn, " Robyns Bush Food".) Given that as a group they are fairly robust and not too particular about growth conditions the *Prostanthera* would appear to be able, potentially, to support a large market once this has been developed. However, even before the development of a market, the safety of these plants for human consumption must first be established. Then the stability of their most prized characteristic, their aroma/ fragrance, must be investigated in order to determine likely shelf life. Finally commercial users should have some means whereby the particular species that are targeted as raw materials can be readily distinguished from other species.

Anti-microbial activity had been reported in the essential oils derived from a number of species of *Prostanthera* ♦ leading to the speculation that the inclusion of the dried powdered leaves in foodstuffs could act as a complete or partial substitute for preservatives in the finished product. For this reason microbiological studies were included in this work. Obviously the allure of *Prostanthera* is increased if by its inclusion, the addition of " un-natural, chemical preservatives "with the attendant spectres of allergies, systemic poisoning, and chemical contamination is rendered unnecessary or to a lowered dosage.

The three species selected for this study were *Prostanthera rotundifolia, Prostanthera lasianthus*, and *Prostanthera incisa*.

The first two are found throughout the southern cooler temperate regions of Australia where they are commonly grown as windbreaks.
The *Prostanthera incisa* (Ballinyah clone) studied came from the Lismore region of northern NSW.

These three species were selected for investigation because they are already grown in significant amounts as crop production, and so ample quantities were available for experimental use. Moreover, *Prostanthera rotundifolia* and *Prostanthera incisa* have already undergone a measure of commercial exposure. They are at present included as a minor constituent of certain products by manufacturers of bushfoods.

It is hoped that from this study the results that emanate will be of value to the native food / culinary industry and thereby increasingly realise the market potential of *Prostanthera.*

Dellar et al, Phytochemistry 36 page 957, 1994.
Also, personal communication from R. Hayes, School of Safety Science, UNSW.

Brief Outline of the Report

This project is made up of six self contained investigations, each employing a different methodology and having its own aims.

1. Description of microscopic and macroscopic features of the collected *Prostanthera* species ---- with the aim of presenting a set of physical conditions that can be used as guidelines in the rapid identification of the whole and powdered herbs –Section B.

2. Description of their sensory properties when in the fresh state, after storage, and after treatments that mimic cooking procedures - ---from this the shelf lives and possible culinary applications can be postulated – Section C.

3. Chemical profiling by means of thin layer chromatography (TLC) of oils and extracts prepared from the herbs at two time intervals – Section E. TLC is a quick and comparatively cheap method of exposing the chemical composition of solutions. By this method not only may the major chemicals present be determined but also the approximate quantities present, thereby yielding some insight into stability and shelf-life. In addition, the TLC band patterns are chemical profiles which can serve as an extra means of rapid identification.

4. Gas chromatography-mass spectrometry (GCMS) identification and quantitation of the principal components of their oils----Section F.
   The results will complement and extend the data from TLC. Having first identified the chemical constituents of the oils, the toxicity of each chemical can then be individually researched to draw up a generalised picture of the consumer safety.

   In addition, the composition of the oils at initial time and post five months should quantify oil stability.

   Furthermore, no detailed GCMS investigations on the oils of *Prostanthera rotundifolia* or *Prostanthera lasianthus* have yet been published. The results from this work will, hopefully, form the beginnings of a bank of data which includes such information as the relation between oil composition and oil quality, and between oil composition and growth conditions.

5. Microbiological determination of antimicrobial activity of the oils--- Section G.
   Through this study the anti-microbial activity of the oils and extracts will be clarified and thus also their role as a natural preservative in foods.

6. A survey of relevant literature focussed on the chemicals defined by GCMS, from which a picture of the safety of the *Prostanthera* specimens may be drawn, Section H.

   The TLC, GCMS and microbial work were extended to include samples that had been dried and stored for a period of five months as well as fresh undried and freshly dried specimens, in order to provide information relating to stability. A longer storage time period would have been desirable, but was not possible under the project boundaries.

2. Sample Pretreatment

Fresh specimens of *Prostanthera rotundifolia* and *Prostanthera lasianthus* were used within 24 hours of collection; the *Prostanthera incisa* specimen (which was despatched from Northern NSW) was used within 48 hours of collection.
All the herbs were inspected upon arrival and found to be in excellent condition.

Specimen verification

Fresh samples were sent to the Herbarium and their identities were verified (please refer to certificate of identification from the Royal Botanic Gardens Melbourne).

Specimen drying

The major portion of the samples were dried in a commercial plant dryer, in a well ventilated atmosphere at temperatures of below 35 °C, *Prostanthera rotundifolia* and *Prostanthera incisa*, being small leaved and finer branched, required about 36 hours for complete drying, whereas *Prostanthera lasianthus* was left in the dryer for about 48 hours.

mass change upon drying:	*Prostanthera rotundifolia*	-59.3 %
	Prostanthera lasianthus -	54.0%
	[*Prostanthera incisa*	data not available]

The dried herbs were stored in large plastic bags, at ambient temperature.

3. Macroscopic and Microscopic Features of *Prostanthera* Specimens

Prostanthera rotundifolia (collected from the Kardella region in South Gippsland)
The aerial portion of the plant appeared to be openly branched, and strongly aromatic. The branches were glabrous, the branchlets moderately covered with short curled hairs, and moderately to densely covered with subsessile glands. The leaf arrangement was opposite, the leaves themselves being ovate to subovate, within the range of 5 – 21 mm long, 5 – 15 mm wide,♦ mid-green and paler in colour below, glabrous except for the midrib which was sparsely hairy. The leaf base was long –attenuate and decurrent, the leaf margin entire or irregularly lobed, the apex was rounded.

The chief microscopic features were sparse thick-wall hairs (basally and along lower end of midrib of abaxial surface), stomal complexes, and sinuate and beaded cell walls. Numerous calcium oxalate crystals were observed in mesophyll cells (see photographs entitled *Prostanthera rotundifolia*.))

Prostanthera incisa (collected from near Lismore, Northern NSW;)
The aerial portion of the plant appeared to be openly branched, and strongly aromatic. The branches were laterally ridged, moderately to densely covered with short curled hairs, the tips being moderately densely covered with subsessile glands. The leaf arrangement was opposite, the leaves were ovate, within the range of 3 - 8 mm long, 2 - 6 mm wide ♦, green (paler below) , and covered with short curled hairs basally, the leaf base was long –attenuate and decurrent. Along the midrib of abaxial surface and leaf margin were situated dense subsessile glands; the leaf margin was coarsely toothed and the leaf apex obtuse.

Microscopically, thick walled hairs were situated basally on each leaf, basally, along abaxial surface and along the margins. Some features were: anisocytic stomatal complexes, subsinuate to sinuate cell walls, numerous calcium oxalate crystals in palisade mesophyll and spongy mesophyll (see photographs entitled *Prostanthera incisa*.)

Prostanthera laisanthus (collected from the Kardella region, South Gippsland)
The aerial portion was openly branched and mildly aromatic. The branches and branchlets were glabrous and densely covered with subsessile glands. The leaf arrangement was opposite, the leaves themselves being ovate to lanceolate, generally within the ranges of 40 – 70 mm long, 5 –25 mm wide, mid-green in colour and paler on the lower surface. The leaves were glabrous, with cuneate to shortly –attenuate base, toothed margined, and the apex acute to acuminate.

Microscopically the leaves bore anomocytic or anisocytic stomatal complexes; the cell walls were sinuate and beaded on the leaf lower surface but were thickened on the leaf upper surface. Numerous rectangular calcium oxalate crystals were present in mesophyll cells (see photographs entitled *Prostanthera lasianthus*.)

♦ These leaf sizes of both *Prostanthera incisa* and *Prostanthera rotundifolia* were at slight variance with that described in " Flora of Victoria vol 4" ed. N.G. Walsh and T.J. Entwisle; the leaves of *Prostanthera incisa* that were examined being smaller than described. This variation was attributed to variation in growth conditions.

Conclusion
Prostanthera incisa and *Prostanthera rotundifolia* were not greatly dissimilar in macroscopic morphology (nor in TLC profiles, see later). While the specimen of *Prostanthera rotundifolia* studied here possessed, overall, larger leaves than the specimen of *Prostanthera incisa* leaf size could be determined by growth conditions and is not therefore a constant determinant of identity.
Prostanthera incisa could however be casually distinguished from *Prostanthera rotundifolia* by its leaf shape, in particular the leaf margin which was more toothed than the latter.
Prostanthera lasianthus was quite distinct from the other two species having larger and longer leaves. Moreover its calcium oxalate were laid as elongulated prisms instead of rosettes.

4. Sensory Observations

The specimens were examined by a panel of six persons and their judgements relating to the smell, texture and taste of the specimens recorded. The specimens were examined as fresh herbs, after drying, and after being subjected to cooking conditions ie microwaveing and baking.

Prostanthera rotundifolia leaves were soft and pliable when fresh. They exuded a strong but pleasant mint / eucalyptal fragrance which became more intense when the leaves were crushed. Very bitter to the taste when chewed, they leave behind however an after taste that was fresh and cleanly astringent.

The mint aroma from *Prostanthera incisa* leaves, which were smaller and delicate, was as powerful as that from the *Prostanthera rotundifolia* but somehow more attractive, including a softer, milder, sweet component. The leaves were less bitter and left a very pleasant after taste.

Prostanthera lasiathus leaves were slightly leathery; their mint / eucalypt smell was less intense than *Prostanthera rotundifolia* or *Prostanthera incisa* but the bitterness was just as strongly off-putting. There was a similarly pleasant aftertaste.

After subjection to microwave all three *Prostanthera* leaves retained remnants of their aroma, and the bitterness was lessened.

Baking caused the three *Prostanthera* leaves to char and lose their fragrance completely.

The aerial terminal portions were dried and then stored in large plastic lined plastic bags of loose weave, at ambient temperature. After a period of just over five months, *Prostanthera incisa* and *Prostanthera rotundifolia* have lost part of their original aroma, but the residual fragrance was still clearly perceptible. *Prostanthera lasianthus* on the other hand had entirely lost its original odour. The bitterness of the leaves was slightly moderated with storage, but was still potent in all three species.

The oils of the specimens were also subjected to the taste test; all three oils tasted bitter, but not as bitter as expected considering that a drop (0.1ml) of the oil was equivalent to chewing about two whole teaspoonfuls of dried crushed leaf.

5. Preparation of Test Samples

D1. Preparation of the essential oils

A portion of the fresh herb leaves and aerial tips were homogenised without pre-drying in a food processor, and allowed to stand in dichloromethane (DCM) for 48 hours, with periodic shaking. The mixture was filtered first through glass wool then filter paper, and the filtrate dried with anhydrous sodium sulphate before being re-filtered and subjected to evaporation under lowered pressure (on a Rotovac) to remove the solvent.

The oil contents were found to be

Prostanthera rotundifolia 4.3 % w/w (dried weight)
Prostanthera lasianthus 1.0 % w/w " "
Prostanthera incisa 3.5 % w/w " " ,

calculated based on the assumption that fresh *Prostanthera incisa* leaves should be of the same moisture content as *Prostanthera rotundifolia* a not unreasonable assumption to make since the leaf sizes and leaf texture of the two species were very similar.

It should be noted that these figures were only approximate because the specimens were damp with dew when received and extracted.

The oils from *Prostanthera rotundifolia* and *Prostanthera incisa* were heavy dark green viscous liquids whereas that from *Prostanthera lasianthus* was a green solid at room temperature. All three were strongly aromatic.

The oils were prepared in the same manner from the dried leaves after 5 months of storage, the step of dehydrating with sodium sulphate being omitted.
The oil yields were:

Prostanthera rotundifolia	4.7% w/w
Prostanthera lasianthus	1.4 % w/w
Prostanthera incisa	3.8% w/w

and were of the same order as the figures for the fresh herbs ,indicating that little had been lost after five months storage.

D2. Preparation of aqueous extracts from fresh herbs

Since a popular method of using *Prostanthera* in food is in the form of an essence prepared by steeping ground fresh leaves (private communication from S. Hess - Buschmann). A herbal extract was prepared by soaking homogenised fresh *Prostanthera* leaves in boiling water at a ratio of 50g leaves per 100 ml water for 48 hours, filtering, and using the filtrate for microbiological tests.

D3. Preparation of extracts from dried herbs

Extracts were prepared in DCM, and in methanol; lipophilic constituents were expected to be extracted by the first solvent while methanol would extract more polar chemicals.

The methanolic extracts were prepared by refluxing 1g of the dried ground leaves to 10 ml methanol, for 15 min. The mixture was cooled and filtered.
DCM extracts were prepared by shaking 1g of the ground dried leaves in 10 ml DCM for 15 min and filtering. The extracts thus obtained were shown by TLC to be similar in composition to the oils. For the purpose of quick chemical profiling, the preparation of an extract instead of the oil would be much more expedient.

The DCM and methanolic extracts were prepared from *Prostanthera* leaves that were dried soon after arrival, and from the dried leaves after 5 months of storage.

D4. Subjecting the herbs to simulated cooking conditions

a. microwave
Dried leaves were subjected to 2 min on “high” setting in a Panasonic convection/microwave oven (Matsushita Electric Industrial Co. Ltd, output 700W, 2450 MHz), then ground and extracted as above.

b. oven baking
Dried leaves were left in an oven set at 180 C for 30 min; then ground and extracted as above.

6. Thin Layer Chromatograph Profiling

The high performance version of TLC was used as a broad chemical screen of the constituents present in the oils and extracts. By varying the composition of the developing solvent systems and by using different visualisation agents, conclusions may be drawn as to the presence/ absence of different classes of chemicals. At the same time, the pattern of the thin layer bands can act as a finger –print of each plants identity.

In this study the plates were HPTLC 10x10 cm Kieselgel 60 F254, glass, from Merck. The chromatography was carried out using an automatic Camag Linomat IV applicator, Camag horizontal Developing Chamber, and Camag automatic Immersion Device. Photographs of the developed plates were taken on a High Resolution Digital Colour /mono camera system equipped with Pixera Visualisation Software and Zoom lens, mounted above a Dual wavelength 255,360 nm UV and white light transilluminator.

E1. TLC of lipophilics

The oils (dissolved in DCM) and DCM extracts were developed in toluene: ethyl acetate (93: 7) and visualised under white light after dipping in vanillin / sulphuric acid reagent and heating to 105 C for 10 min.

Linalool, cineole, and globulol were spotted on some of the plates to screen for the presence of these chemicals in the oils.

The following conclusions were drawn

- The bands exhibited by the three oils were broadly similar, Fig 1, indicating that a number of chemicals possibly occurred in all three species, for example the chemicals corresponding to the bands at 25,and 35 mm from the baseline.
    - The faintness of the *Prostanthera lasianthus* bands indicated that its oil contained only low concentration of these two and other compounds; and this is one of its distinguishing characteristic from the other two members.
    - *Prostanthera rotundifolia* may be distinguished from *Prostanthera incisa* which it closely resembles in TLC profile, by its topmost major band at 50 mm which was not evident in the latter, and by the absence of a major band at 20 mm which was present in *Prostanthera incisa*
    - While the cineole band was observed in all three oils albeit just barely perceivable in *Prostanthera incisa*, linalool on the contrary, was absent from all three oils; the globulol band was clearly perceptable in the oil of *Prostanthera incisa*., Fig. 2,3,4.
    - Apart from the band at 40mm of *Prostanthera rotundifolia* fresh oil (which was very much weaker in the oil after 5 months storage Fig.2, and Fig.5), all other major bands in the fresh and 5 months oils were essentially identical, indicating a measure of chemical stability within this time period.
    - Microwaving did not destroy the major chemicals in the oils; however baking destroyed all the constituents which were detected by TLC , Fig.6

E2. TLC of more polar constituents

a. TLC of flavonoids and phenol carboxylic acids

Methanolic extracts were applied onto plates which subsequently were developed in ethyl acetate / formic acid / acetic acid /water 100:11:11:27.The dried plates were dipped in a methanolic solution of 1 % diphenylboric acid B ethylamino ester and 5 % polyethylene glycol 4000, dried, and inspected under light of 365 nm.

Under these conditions flavonoids are characterised by orange-yellow to yellow-green fluorescence, whereas the presence of phenol carboxylic acids are indicated by blue-white fluorescence.

All three species contained flavonoid compounds which were neither rutin nor hyperoside, Fig.7, and while the presence of phenol carboxylic acids was intensely obvious in all of the extracts, only weak bands at about 50 mm suggested the presence of chlorogenic acid. The caffeic acid band could not be seen. [Note: the distances in Figure 7 were measured from the bottom edge of the plate.]

The band patterns of *Prostanthera rotundifolia* and *Prostanthera incisa* were similar, but *Prostanthera lasianthus* differed by displaying an orange band at 80mm, and by the absence of the orange doublet at about 25mm Fig 7.

The close similarity between *Prostanthera rotundifolia* and *Prostanthera incisa* was further illustrated by Fig.8. Here microwaving was also shown to have no effect upon the bands, unlike baking.

The lack of change in band appearance suggested once again some stability of the polar constituents within a 5 month period, Fig. 9.

b. TLC of alkaloids

A screen for alkaloids using methanolic extracts developed in toluene: ethyl acetate: diethylamine 70: 20: 10 and sprayed with Dragendorff reagent ♦ was negative.

[♦ Dragendorff reagent : a mixture of basic bismuth nitrate and potassium nitrate in acetic acid solution]

c. TLC of terpenoids / bitter principals

The methanolic extracts were developed in ethyl acetate; methanol; water 77 : 15 : 8;bands were detected through dipping in vanillin / sulphuric acid reagent and the application of heat, Fig.10

Prostanthera lasianthus was clearly defined from the other two by the presence of three strong bands; the similarity in band pattern between *Prostanthera rotundifolia* and *Prostanthera incisa* was once again obvious.

E3. Summary

Three TLC systems are suitable for *Prostanthera* extracts:

ethyl acetate / formic acid/ acetic acid /water and diphenyl boric acid ester (for methanolic extracts);

ethyl acetate / methanol / water and vanillin-sulphuric acid (for methanolic extracts);

toluene/ ethyl acetate and vanillin –sulphuric acid (for DCM extracts and DCM solution of the oils).

Cineole and globulol may be usefully spotted with the oil samples as references.

7. GCMS Identification and Quantitation of the Major Oil Components

F1. materials and method

Extracts and oils prepared as given previously were diluted before analysis on a gas chromatograph GC: Shimadzu GC17A which included a DB1 capillary column, 0.25 mm internal diameter, 30 m long, connected to an mass spectrometer Shimadzu QP 5000, and auto-injector AOC-20i. The carrier was helium, and the GC experimental conditions were:

Split ratio 1 : 50
Injected volume 1:L
Injection port temperature: 250 C

Temperature program: initially 50 C (0.5 min), then raise at rate of 5 deg. / min to a final temperature of 250 (3 min before re-cycling).
Flow (in terms of kPa): initially, 24.5 for 0.5 min and then increase at rate of 1.7 / min to 91.5 (held for 3.6 min and then recycle).

The chromatographic peaks were identified through injecting solutions of pure chemicals and comparing retention times and mass spectra. When this was not possible identification was tentatively decided through computer comparison of the mass spectra of the samples with those in the reference libraries: NIST 12 and NISTC 64 (both ex Shimadzu). Such assigned identifications are characterised in the Tables below by their Similarity Index SI, an Index of 100% being parallelled by a similar certainty in identification. Quantitation of some of the major components was carried out when the pure chemicals were available, through establishing area/concentration calibration curves (for example Figure F.1) and applying these to sample areas.

F2 GCMS results

The Tables below summarise the chemicals found, the proportion of each chemical in the sample (indicated approximately by its peak area expressed as % of total peak areas) and the concentration of some of the chemicals in terms of μL pure substance / ml oil or / ml extract.

a. *Prostanthera rotundifolia*
[please refer Tables F.1.1 to F.1.5]

There were three components in each of the oils and extracts for which no matches could be found. The three components, numbers 10,11,12 of Table F.1.1 had the same retention times as components 4,5,and 6 of Table F.1.3, which were 22.6min, 24.2 min, and 24.5 min. Components 4,5,6 of Table F.1.2 and Table F.1.4 eluted at times 22.3min, 23.9 min and 24.2 min. It seemed likely that the compound of retention time 23.9 min was detected only after storage or microwaving. One of the unidentified components, of retention time 22.3 – 22.6 min, appeared to make up a large proportion of the oils and extracts (area % about 30 to 40 of the total); the other unidentified components were present in lower but still significant quantities.

Future investigations should centre on discovering the identities of these unknown but important components.

The principal component of the oils and DCM extracts was cineole, its level present in the oils was shown to drop dramatically with storage. Baking appeared to destroy or caused the complete loss of all oil components, for all three *Prostanthera* species.

b. *Prostanthera incisa*
[please refer Tables F.2.1 to F.2.5]
The oils of *Prostanthera incisa* included most of the components that appeared in the oils of *Prostanthera rotundifolia*, plus extras, chief of which were linalyl acetate, terpinyl acetate, and globulol. Cineole was again the principal chemical making up the oil but at a slightly lower concentration in terms of μL/ ml oil. Quantitation of the components proved that there was a moderate loss of cineole, pinenes, and globulol with storage.

The results of this analysis were broadly similar to that communicated to us by S. Hess Buschmann. However the chemicals limonene, myrcene, terpineol, and linalool -- identified as major components (peak area > 1% of total) were not found in this study. The absence of linalool from *Prostanthera incisa* oil was confirmed by our TLC results, see Section E.

c. *Prostanthera lasianthus*
[please refer to Tables F.3.1 to F.3.5 here]
Cineole was once again the component that made up the largest proportion of the oil, dropping precipitately from 170.5 μL /ml fresh oil to only 22.1 μL /ml of oil, from the stored specimen.

From non-MS analysis using GC, Lassak reported globulol in *Prostanthera incisa* oil, globulol and cineole in the oil of *Prostanthera rotundifolia* and cineole and at times linalool in *Prostanthera lasanthius* especially specimens from Mt. Tomah (μL assak, E. 1980, 8th International Congress Essential Oils, Cannes/Grasse.) In the light of this and recalling the statement concerning *Prostanthera incisa* above, it may be concluded that possibly the oil composition varies with the growth conditions. This is an interesting probability and merits further investigation.

d. *a-pinene, b- pinene, cineole, and globulol in Prostanthera leaves*
From their concentrations in the oils (μL / ml), and knowing the oil content of the leaves (see Section D) the approximate concentrations of four components in the dried leaves were calculated (as μL / 100g dried leaf)

Table F.4 Levels of chemicals in *Prostanthera* dried leaves

Prostanthera	cineole	a-pinene	b-pinene	globulol
P. rotund. fresh	890	13	15	0
P. rotund. 5 months	193	0	0	0
Prostanthera incisa fresh	577	7	14	298
Prostanthera incisa 5 months	478	2	4	108
P. lasian., fresh	136	14	2	0
P.lasian. 5 months	31	2	1	0

Of the three oils, the oil of fresh *Prostanthera rotundifolia* had the highest level of cineole, which level dropped steeply upon storage. The cineole content in the oil of *Prostanthera incisa* on the other hand was more constant with time.

8. Microbiological Investigations

The three species of *Prostanthera* were tested for anti-microbial activity as oils (as oil dissolved in DCM in the case of *P. lasianthus*) and as extracts in water, methanol, and DCM. These oils and extracts were prepared at time zero and from the dried stored herbs (for details see Section D).

G1 Antimicrobial Activity Testing

G1.1 Bacterial Cultures Used in Antimicrobial Activity Testing

The following five bacterial species were used for antimicrobial testing: *Staphylococcus aureus* (ATCC 29213); *Escherichia coli* (ATCC 25922); *Pseudomonas aeruginosa* (ATCC 27853); *Erwinia carotovora* subs *P. carotovora* (Accession No. not available); and *Bacillus subtilus* (Accession No. not available). Before antimicrobial testing began each bacterial species was cultured at 35^{o}C overnight in nutrient broth (Oxoid, Melbourne), then swab inoculated onto Sensitest agar (Oxoid, Melbourne) plate, and incubated at 35^{o}C overnight to ensure that an even lawn was obtained.

G1.2 Prostanthera Oil Extracts Antimicrobial Activity Testing Method

Each bacterial species was prepared as a 35^{o}C overnight culture in nutrient broth (Oxoid, Melbourne), then a Sensitest agar (Oxoid, Melbourne) plate was swab inoculated with the overnight culture, and a filter paper disc was placed onto the Sensitest agar plate (Oxoid, Melbourne). The *Prostanthera* oil extract to be tested was then applied to the disc. In each case a 10 μL volume was used, and allowed to absorb into the disc and the surrounding agar before incubation of the plate overnight at 35^{o}C. The result of the test was recorded by measuring any observable zone of inhibition caused by the extract.

G1.3 Solvent Antimicrobial Activity Testing of Method

Control plates were prepared to test the antimicrobial activity of the solvents, methanol and dichlormethane, used in two of the extraction procedures. These tests were prepared using the same method as used for the *Prostanthera* oil extracts (Section G1.2). In each case 10 μL of the solvent was applied to the disc, and the results were recorded as observed zone of inhibition.

G1.4 Antibiotic Positive Controls Antimicrobial Activity Testing Method

Tetracycline (30 g/disc) and Ciprofloxacin (2.5 g/disc) (Oxoid, Melbourne) antibiotic discs were applied to agar plates swab inoculated with the five bacterial species. This test was prepared using the method outlined in Section G1.2, except that the prepared antibiotic discs were used instead of blank discs with test substances added. The result was recorded as observed zone of inhibition.

G1.5 Results of Prostanthera Antimicrobial Activity Test

All five species of bacteria used in the antimicrobial activity testing showed an even lawn of growth after incubation at 35^{o}C overnight on the Sensitest agar used in this experiment.

The results in the following tables are recorded as the zone of inhibition measured in millimetres from the edge of the filter paper disc to the point where bacterial growth is again observed. Where direct contact inhibition is recorded this refers to the inhibition of growth resulting from direct contact of the test substance with the agar plate due to overflow of sample from the filter paper disc. The results reported are qualitative only, and not quantitative.

Table G1.1 Antimicrobial Activity of Tetracycline (30 g/disc) and Ciprofloxacin (2.5 g/disc) versus the Bacterial Species used in *Prostanthera* Oil Extract Tests – Results Recorded as Zone of Inhibition in Millimetres

Bacterial Species	Zone of Inhibition caused by Tetracycline (30mg/disc)	Zone of Inhibition caused by Ciprofloxacin(2.5mg/disc)
Escherichia coli	5 mm	10 mm
Pseudomonas aeruginosa	6 mm	12 mm
Erwinia carotovora subs *P. carotovora*	6 mm	3 mm
Staphylococcus aureus	10 mm	8 mm
Bacillus subtilus	8 mm	10 mm

Table G1.2 Antimicrobial Activity of Methanol and Dichlormethane versus the Bacterial Species used in *Prostanthera* Extract Tests – Results Recorded as Zone of Inhibition in Millimetres

Bacterial Species	Zone of Inhibition caused by Methanol	Zone of Inhibition caused by Dichlormethane
Escherichia coli	1 mm direct contact	1 mm
Pseudomonas aeruginosa	1 mm direct contact	1 mm
Erwinia carotovora subs *P. carotovora*	1 mm direct contact	1 mm
Staphylococcus aureus	0 mm	1 mm
Bacillus subtilus	0 mm	1 mm

Table G1.3 Antimicrobial Activity of Time Zero H_2O Extracts of Three *Prostanthera* Species – Results Recorded as Zone of Inhibition in Millimetres

Bacterial Species Used to Test the Antimicrobial Activity of *Prosanthera* Species H_2O Extracts	Zone of Inhibition caused by *Prostanthera incisa* H_2O Extract	Zone of Inhibition caused by *Prostanthera rotundifolia* H_2O Extract	Zone of Inhibition caused by *Prostanthera lasianthus* H_2O Extract
Escherichia coli	0 mm	0 mm	0 mm
Pseudomonas aeruginosa	0 mm	0 mm	0 mm
Erwinia carotovora subs *P. carotovora*	0 mm	0 mm	0 mm
Staphylococcus aureus	0 mm	0 mm	0 mm
Bacillus subtilus	0 mm	0 mm	0 mm

Table G1.4 Antimicrobial Activity of Time Zero Methanol Extracts of Three *Prostanthera* Species – Results Recorded as Zone of Inhibition in Millimetres

Bacterial Species Used to Test the Antimicrobial Activity of *Prosanthera* Species Methanol Extracts	Zone of Inhibition caused by *Prostanthera incisa* Methanol Extract	Zone of Inhibition caused by *Prostanthera rotundifolia* Methanol Extract	Zone of Inhibition caused by *Prostanthera lasianthus* Methanol Extract
Escherichia coli	1 mm direct contact	0 mm	0 mm
Pseudomonas aeruginosa	0 mm	0 mm	0 mm
Erwinia carotovora subs *P. carotovora*	0 mm	0 mm	0 mm
Staphylococcus aureus	0 mm	0 mm	0 mm (2 mm halo of lighter growth)
Bacillus subtilus	0 mm	0 mm	0 mm

Table G1.5 Antimicrobial Activity of Time Zero Oils of Three *Prostanthera* Species – Results Recorded as Zone of Inhibition in Millimetres

Bacterial Species Used to Test the Antimicrobial Activity of *Prosanthera* Species Oil	Zone of Inhibition caused by *Prostanthera incisa* Oil	Zone of Inhibition caused by *Prostanthera rotundifolia* Oil	Zone of Inhibition caused by *Prostanthera lasianthus* Oil
Escherichia coli	0 mm	0 mm	1 mm direct contact
Pseudomonas aeruginosa	0 mm	0 mm	1 mm direct contact
Erwinia carotovora subs *P. carotovora*	0 mm	0 mm	0 mm
Staphylococcus aureus	3 mm	3 mm	2 mm
Bacillus subtilus	4 mm	3 mm	0 mm

Table G1.6 Antimicrobial Activity of Time Five Months Methanol Extracts of Three *Prostanthera* Species – Results Recorded as Zone of Inhibition in Millimetres

Bacterial Species Used to Test the Antimicrobial Activity of *Prosanthera* Species Methanol Extracts	Zone of Inhibition caused by *Prostanthera incisa* Methanol Extract	Zone of Inhibition caused by *Prostanthera rotundifolia* Methanol Extract	Zone of Inhibition caused by *Prostanthera lasianthus* Methanol Extract
Escherichia coli	1 mm direct contact	1 mm direct contact	0 mm
Pseudomonas aeruginosa	0 mm	0 mm	0 mm
Erwinia carotovora subs *P. carotovora*	1 mm direct contact	1 mm direct contact	0 mm
Staphylococcus aureus	1 mm direct contact	1 mm direct contact	0 mm
Bacillus subtilus	0 mm	0 mm	0 mm

Table G1.7 Antimicrobial Activity of Time Five Months Oil of Three *Prostanthera* Species – Results Recorded as Zone of Inhibition in Millimetres

Bacterial Species Used to Test the Antimicrobial Activity of *Prosanthera* Species Oil	Zone of Inhibition caused by *Prostanthera incisa* Oil	Zone of Inhibition caused by *Prostanthera rotundifolia* Oil	Zone of Inhibition caused by *Prostanthera lasianthus* Oil
Escherichia coli	0 mm	0 mm	2 mm direct contact
Pseudomonas aeruginosa	1 mm direct contact	0 mm	1 mm direct contact
Erwinia carotovora subs *P. carotovora*	0 mm	0 mm	1 mm direct contact
Staphylococcus aureus	3 mm	2 mm	1 mm direct contact
Bacillus subtilus	4 mm	3 mm	1 mm direct contact

Table G1.8 Antimicrobial Activity of Time Five Months Dichlormethane Extracts of Three *Prostanthera* Species– Results Recorded as Zone of Inhibition in Millimetres

Bacterial Species Used to Test the Antimicrobial Activity of *Prosanthera* Species Dichlormethane Extracts	Zone of Inhibition caused by *Prostanthera incisa* Dichlormethane Extract	Zone of Inhibition caused by *Prostanthera rotundifolia* Dichlormethane Extract	Zone of Inhibition caused by *Prostanthera lasianthus* Dichlormethane Extract
Escherichia coli	1 mm direct contact	1 mm direct contact	1 mm direct contact
Pseudomonas aeruginosa	1 mm direct contact	1 mm direct contact	1 mm direct contact
Erwinia carotovora subs *P. carotovora*	1 mm direct contact	1 mm direct contact	1 mm direct contact
Staphylococcus aureus	1 mm direct contact	1 mm direct contact	1 mm direct contact
Bacillus subtilus	1 mm direct contact	1 mm direct contact	1 mm direct contact

G1.6 Discussion

Antimicrobial activity was observed when using the oils of *Prostanthera incisa* and *Prostanthera rotundifolia* from both the Time Zero and Time Five Month samples, however, this activity was only observed against the Gram positive bacterial species, *S. aureus* and *B. subtilus*. *Prostanthera incisa* and *Prostanthera rotundifolia* showed no antimicrobial activity against the remaining bacterial species, all of which were Gram negative organisms. No antimicrobial activity was observed against any bacterial species when testing the *Prostanthera lasianthius* oils or when testing any of the extracts, indicating that (1) the displayed anti Gram positive bacterial activity probably originated in the lipophilic components and (2) the activity may be concentrated related. *Prostanthera lasianthius* oil was tested solubilised in DCM because of the solid state of the pure oil. In this and in the extracts the lipophilic components were present at much lower levels or were absent altogether.

G2 Preservative Activity Testing

G2.1 Prosanthera Preservative Activity Testing Method

Dried plant material from time zero samples was steeped 1:1 in distilled water, 10% sucrose solution, or a commercial brand of canola oil, and stored in closed containers for 4 months at room temperature. After four months these containers were opened and used as a source of inoculum. Duplicate samples were streak inoculated onto Sensitest agar (Oxoid, Melbourne) plates, and incubated at 35^{o}C overnight, or room temperature for one week. After these times the plates were observed visually for any sign of microbial growth, and the result recorded. Both sets of plates were then incubated at room temperature for a further one month, and again visually inspected for any sign of microbial growth.

The results of these tests are recorded as Growth or No Growth in the following tables. No attempt was made to identify the microorganisms observed on the plates, however, by visual inspection it could be seen that bacterial, yeast and fungal species were present.

Table G2.1 Microbial Contamination Observed in Distilled Water, 10% Sucrose in Distilled Water, and Canola Oil after Four Months Storage at Room Temperature in Closed Containers – Results Recorded as Growth or No Growth

Solution kept for Four Months at Room Temperature	Observation after Incubation at 35°C Overnight	Observation after Incubation at Room Temperature for One Week	Observation after Incubation at Room Temperature for One Month
Distilled water	No Growth	No Growth	Growth
10 % sucrose in distilled water	No Growth	No Growth	Growth
Canola oil	No Growth	No Growth	Growth

Table G2.2 Microbial Contamination Observed in Preparations of Three *Prostanthera* Species Steeped in either Distilled Water, or 10% Sucrose in Distilled Water, or Canola Oil after Four Months Storage at Room Temperature in Closed Containers – Results Recorded as Growth or No Growth

Prosanthera Species and Solution in which it was Steeped for Four Months at Room Temperature	Observation after Incubation at 35°C Overnight	Observation after Incubation at Room Temperature for One Week	Observation after Incubation at Room Temperature for One Month
Prostanthera incisa in Distilled Water	No Growth	No Growth	Growth
Prostanthera rotundifolia in Distilled Water	No Growth	No Growth	Growth
Prostanthera lasianthus in Distilled Water	No Growth	No Growth	Growth
Prostanthera incisa in 10 % Sucrose Solution	Growth	Growth	Growth
Prostanthera rotundifolia in 10% Sucrose Solution	No Growth	No Growth	Growth
Prostanthera lasianthus in 10 % Sucrose Solution	No Growth	No Growth	Growth
Prostanthera incisa in Oil	No Growth	No Growth	Growth
Prostanthera rotundifolia in Oil	No Growth	No Growth	Growth
Prostanthera lasianthus in Oil	No Growth	No Growth	Growth

G2.2 Discussion

Some incipient preservative action was observed for all *Prostanthera* species. No growth was observed after incubation at 35°C overnight or room temperature for one week, except in one sucrose solution, however, the preservative activity was only temporary with growth being observed after incubation for one month at room temperature in each test sample. This result indicates that bacteriostatic activity, bacterial and fungal spores or vegetative structures were still present in the *Prostanthera* samples when they were subcultured onto the agar plates, rather than a bacteriocidal activity was responsible for the preservative action. The limited preservative activity observed in this test will need to be further investigated to establish the usefulness of *Prostanthera* as a natural preservative compound.

In summary, weak anti-bacterial action was observed in the oils of the *Prostanthera rotundifolia* and *Prostanthera incisa* and this was still present at five months. Ground dried leaves were found to possess some but limited preservative ability.

Assessment of whether the oils demonstrated bacteriostatic or bacteriocidal action was not carried out in this study because of time constraints. The results of the preservative investigation indicated that weak bacteriostatic activity was present in the leaves.

9. The Effects of Human Ingestion of the Three Pros. Species from a Survey of Available Literature

The major chemicals (defined as having peak areas > 1% of total area in a GC chromatogram) found in this and another study (commissioned by S. Hess-Buschmann) in the three species were

Cineole
a- and b- pinene
sabinene
myrcene
a phellandrene
limonene
p-cymene
linalool
linalyl acetate
globulol
terpinyl acetate.
b-elemene
germacrene

The principal records that were searched for information relating to physiological / biological effects of the above list upon humans and, in the absence of human data, upon mammalian tissues, were

- Material Safety Data Sheets MSDS compiled by Sigma Aldrich Ltd ,
- Biological Abstracts, 1980 to Sept. 1999. [Biological Abstracts is a publication which provides comprehensive coverage of major international biological and biomedical journal literature.]
- " Essential Oil Safety a Guide for Health Care Professionals" by R. Tisserand & T. Balacs, Churchill Livingstone, New York, 1995.
- " Side effects of Drugs Annual " ed. Dukes, M. Excerpta Medica, 1977 to 1998.
- " Adverse effects of Herbal Drugs" vol 1,2,3; ed De Smet et al, Springer-Verlag 1992.

With the exception of globulol, b-elemene and germacrene, the MSDS sheets gave the blanket advise that all of the above chemicals were likely to be harmful when inhaled, swallowed, or upon skin contact. The chemicals were furthermore likely to be irritating to the eyes and to the respiratory system. Suitable protective clothing should be worn when handling these substances.

A number of animal studies LD50 were also given. These are summarised in the following Table.

Table 1 The LD 50 of some chemicals found in the oils of *Prostanthera*s

Chemical	**LD50**	**other effects**
Cineole	rat, oral: 3.1 g/ kg Rabbit, skin: > 5g/kg	
limonene	mouse, oral: 5.55 ml/kg	moderate skin irritation, rabbit : 50 mg / 24 hours
p- cymene	rat, oral: 4.75g/kg	
linalool	rat, oral: 2.79 g/kg	
terpineol	rat, oral: 5.17 g/kg	
linalyl acetate	rat, oral: 13.93 g/kg	
terpinyl acetate	rat, oral: 5.07 g/kg	

Below are the results of a survey of the biological effects of each of the chemicals.

Cineole

Non-toxic, non-irritant and non-sensitising [1], cineole has low oral toxicity [2] but ingestion of 1 ml has caused transcient coma. Recovery has occurred after ingestion of 30 ml. Poisoning produces severe gastrointestinal and central nervous system CNS effects. The Council of Europe permits its inclusion in foods at a level below 15 ppm.

Animal studies indicated that cineole can exert more subtle effects. Protein droplet nephropathy developed in male rats after short term dosage [3]; cineole caused weak in vitro inhibition of liver mono-oxygenases, [4]; administered alone or in conjunction with ethanol, cineole was found by different groups to enhance epidermal penetration by drugs [5,6]; in common with a number of other essential oil components, cineole was found to possess anti-virial [7] as well as anti-microbial [8,9] activities. Anti-allergic [10] and cancer preventative [11] properties have also been ascribed to cineole.

There was one case of suspected adverse drug reaction concerning cineole. Cineole in the drug Endrine was suggested to be the cause of an incident of granulomatous lesion (from Reports of Suspected Adverse Drug Reactions number 5, Aug 64 –Sept.80; Australian Drug Evaluation Committee.)

a- and b- pinenes

Both isomers are of low toxicity but swallowing extremely large quantities of a-pinene produces CNS depression, bronchitis and kidney damage. a-pinene can cause the appearance of benign tumours. Whilst it is non-irritating in itself, when oxidised it can cause skin sensitising [12].
In vitro studies demonstrated that a-pinene possesses anti-microbial action [13,14] and that both isomers may affect respiratory function after inhalation [15].
A-pinene was also implicated in interfering with the function of certain rat liver microsomal enzymes [16].

Sabinene

Sabinene was found active against Gram positive bacteria [17] and fungi [18]; it also inhibited protein induced inflammation in rabbit eyes [19].
There was no evidence of toxicity.

Myrcene
Non-toxic, mildly irritant and non-sensitising, doses of up to 250 mg/kg given to rats had no effect upon peri- and post-natal development. Above 500 mg/kg some adverse effect on birth weight, perinatal mortality and postnatal development was noted [20]. In rats the oral LD50 was determined to be 5g/kg [21]. But myrcene showed an ability to induce and inhibit the activities of certain rat liver enzymes [22,23]; it reportedly exerted potent peripheral analgesic effect on the rat [24] and anti-mutagenic capacity on mammalian cells [25].

a-phellandrene
Non-toxic; can be irritating to the skin and is easily absorbed via this route; ingestion can cause vomiting and diarrhoea. It may increase the risk of cancer in those exposed to carcinogens, but is not carcinogenic in its own right [12].
Its strong anti-microbial activity has been documented [8].

Limonene
There is no indication that limonene is toxic to humans [26,27] It reportedly quenched the ability of citral to cause sensitisation when administered together with citral [28].
More recent animal studies have cleared it as a risk factor in cancers of the kidney [29] and of the mammaries [11].
It may possess anti-inflammatory ability [30] and limonene reportedly increased the influx of thyrotropin hormone across human epithelium [31].

p-cymene
Non-toxic, non-sensitising, but a mild irritant [32], cymene showed potent anti-fungal and anti-microbial activity [8].

Linalool
Narcotic effects were seen at about half the lethal dose in mammals ; otherwise non-toxic, non-irritating and non-sensitising [1].
The narcotic effects may assume the form of sedation [33]; other properties displayed by the chemical were anti-fungal [34, 35,36] and as an insect fumigant [37].

Linalyl acetate
No evidence of toxicity was found [38].
In common with linalool, linalyl acetate sedated rats after its inhalation [33].

a- terpineol and terpinyl acetate
Both these chemicals are non-toxic, non-sensitising and at worst only mildly irritant [39,32]
The former may affect liver microsomal cytochrome P450 systems [40].

Globulol
No information about globulol was found in any of the consulted publications.

b-elemene
This had been tested and found effective as an antineoplastic agent, used by itself or in conjunction with other drugs [41,42] Some essential oils which included it as a major component showed inhibition of bacterial and fungal growth [43,44]

Germacrene

This chemical was detected in many essential oils and yet only a few biological studies have been reported. The oil of Clausena, which included germacrene as a major constituent, was demonstrated to have preservative, anti-bacterial and anti-fungal activities [45].

The paucity of relevant data led to the close scrutiny of eucalytus oil, mainly as a comparative reference.

The major component of the oil of most eucalypts is cineole, with phellandrene, limonene, a- and B- pinene, etc as minor constituents---- not too dissimilar from the composition of *Prostanthera*. Eucalypt oil was accorded GRAS status (generally recognised as safe) by the FDA, while the Council of Europe permits the use of the leaf as spice or seasoning. However the undiluted oil was accounted toxic, the fatal dose being about 3.5 ml. A safe adult dose was taken to be 0.06—0.2 ml (Martindale: The Extra Pharmacopoeia, 29th edition,1982.)
On this basis, the innocuous nature of the oils of *Prostanthera* would be expected at the same low doses.

Summary and conclusion

The literature survey turned up little that was alarming about the toxicity of the above chemicals, and so it may be inferred that moderate quantities of the three *Prostanthera* species may be ingested with very low expectations of adverse effects.

By making the following assumptions:

that the specific gravity of the *Prostanthera* oils is 0.9*[*] and

and that the oil content in the leaf is an average of 4g/100g dried leaf, then a dose of 0.2 ml oil is equivalent to 4.5 g dried leaf or four and a half teaspoonfuls of dried ground leaf; ie this is the amount that an adult could freely ingest, assuming parity with Eucalypt oil.

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[*] * The specific gravity of the oil from *Prostanthera rotundifolia* was 0.941, and the oil from *Prostanthera laisanthus*, 0.912 Althofer, G " Cradle of Incense—the Story of Australian *Prostantheras* ", a publication of the Society for Growing Australian Plants, 1978.

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10. Appendices

(Not available in this online version)