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Medicinal compounds, chemically and biologically characterised from extracts of Australian *Callitris endlicheri* and *C. glaucophylla* (Cupressaceae): Used traditionally in Aboriginal and colonial pharmacopoeia

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ABSTRACT

Ethnopharmacological significance: *Callitris endlicheri* and *C. glaucophylla* were highly valued by Australian Aboriginal people for use in medicinal applications. Pine needles were prepared using modalities of either smoking or topical preparations, requiring either aqueous or lipophilic extraction into animal fat. Extracts treated various ailments consistent with pathogenic infection, or other topical or tracheal ailments not clearly elucidated in ethnopharmacological records.

Aim of the study: Here we aim firstly to investigate antimicrobial activities of both smoke, essential oil and solvent extracts and secondly to chemically characterise significant volatile compounds potentially related to medicinal or antimicrobial activities.

Materials and methods: Essential oils were produced using traditional hydrodistillation of pine needles collected from *Callitris endlicheri* and *C. glaucophylla*. From the same material, solvent extracts were produced separately, using acetone and methanol, and then smoke extracts were produced with separate methods described herein, using fresh needles. All extracts were screened for antimicrobial activity against a range of bacterial organisms and sporicidal activity against pathogenic fungi (*Trichophyton mentagrophytes*, *T. interdigitalis* and *T. rubrum*).

Results: Essential oils produced only modest antibacterial activity and the *Callitris endlicheri* essential oil had moderate antifungal activity. Smoke extracts demonstrated considerable broad spectrum antimicrobial activity, but solvent extracts demonstrated more selective activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and the yeast *Candida albicans*. Chemical character of essential oils was consistent with previous studies; however, solvent and smoke extracts from fresh needles produced high concentrations of potentially medicinal abietane diterpenes, specifically pisiferal, pisiferol and ferruginol; well known from Japanese species with demonstrated bioactivity.

Conclusion: The occurrence of these diterpenes and other phenolics, in conjunction with significant antimicrobial activities from the various extracts, is in alignment with the use of Australian *Callitris* species in Aboriginal medicinal practice.

1. Introduction

1.1. Taxonomy

Both *Callitris endlicheri* (Parl.) F.M. Bailey and *Callitris glaucophylla* Joy Thomps and L.A.S. Johnson have the characteristic conical habit of a Christmas tree, particularly *Callitris glaucophylla*, which is often cut down and resurrected in regional Australian homes during the Christmas season. In older age both of these species lose the relative symmetry that once made them aesthetically attractive for this purpose.

Although *Callitris endlicheri* has been a well described species in Australia since the late 19th century (Bailey, 1883), *Callitris glaucophylla* was named only recently (Thompson and Johnson, 1986). Before this *Callitris glaucophylla* was included in the species *Callitris columellaris* F. Muell., which incidentally at that time was variously described as having three variants requiring clarification. This was established in 1986 when *Callitris glaucophylla* was

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named and the other variant, *Callitris columellaris* var *intratropica*, was raised to species rank as *Callitris intratropica* (Thompson and Johnson, 1986). Subsequently, *Callitris columellaris* was re-described and the name is now assigned to the less common coastal variant, where the type locality of the species was first described (Mueller, 1866).

The most common vernacular names used for *Callitris endlicheri* and *Callitris glaucophylla* are Black Cypress and White Cypress respectively. Although the conical habit can aid identification of the respective species, differential classification can be more objectively performed by examining the cones. White Cypress (*Callitris glaucophylla*) can be distinguished from Black Cypress (*Callitris endlicheri*) if the inside of the cone has a single columella structure (spike), as opposed to a three-lobed or three individual columella found within the open cone scales of Black Cypress.

1.2. Traditional uses of Callitris

With regard to traditional use reports of *Callitris columellaris* (or additionally early phytochemical papers describing the same species) by Australian Aboriginal people, a significant number of these accounts may correctly apply to *Callitris glaucophylla* because records were produced before this species name entered the literature. This is particularly likely because *Callitris glaucophylla* occupies the largest geographic range of Australian *Callitris* species. In most ethnobotanic records the origin of plant material collected for studies is described, or the Aboriginal tribal group affiliated with traditional medicinal applications is named. Thus, the correct associated species can be deduced.

Traditional medicinal uses of *Callitris glaucophylla* or *Callitris endlicheri* by Australian Aboriginal people include topical applications to target conditions such as scabies mite, sores or rashes using either aqueous or lipophilic animal fat extracts. Smoke fumigation treatments were also employed using pine needles in a pit over fire embers, with the patient positioned above the pit. Targeted ailments include coughs and colds, or illnesses not described in the literature but apparently resolved by inducing a sweating condition that persisted during sleep, which evidently cleansed the system of toxins related to ill health (Barr, 1988; Clarke, 2007; Isaacs, 2000; Low, 1990; Latz, 2004; Lassak and McCarthy, 2011; Williams, 2011).

The two best known uses of *Callitris endlicheri* and *Callitris glaucophylla* were adopted by early colonial Australians. Firstly, a resinous exudate, sometimes called a resin, gum or tree sap, was collected from injured trees and dissolved in alcohol for subsequent use as an enteric coating for tablets; preventing dissolution in the stomach but promoting digestion upon entry into the small intestine. Low (1990) adds that this resin was also used on occasion to fill a tooth cavity, but it is not clear over what period of time this filling was expected to endure.

This resinous exudate, rich in diterpenoid acids and essential oils, was given the commercial name Sandarac (Cribb and Cribb, 1981; Low, 1990; Lassak and McCarthy, 2011; Williams, 2011). During the Sandarac boom a famous colonial botanist and ethnobotanist, Sir Joseph Henry Maiden (1859–1925), encouraged small family run business initiatives to fuel the industry by employing the collecting efforts of the children, thereby making the industry economically viable (Low, 1990; Williams, 2011). Lassak and McCarthy (2011) add that *Callitris* plantations, normally used for timber, will yield sufficient quantities of the exudate from the cut trunks a year or more after logging. Lassak and McCarthy (2011) go on to say that the subjective experience of fresh air, with a piny aroma, makes this work environment an attractive and pleasant place to be.

The second well-known colonial use of the two *Callitris* species examined here was for termite resistant timber. After it became known that the timber significantly resisted termite feeding, from observation of houses built decades earlier using this respective timber, an attempt was made to establish an export timber industry; but economic viability could not be established due to high transport costs (Maiden, 1889).

1.3. Phytochemical studies

More recently studies have attempted to describe volatile compounds, produced by solvent extraction and hydrodistillation of the wood, which may be associated with termite resistance. Sesquiterpene γ-lactones, columellarin, dihydrocolumellarin; sesquiterpene alcohols, guaiol, β-eudesmol; sesquiterpene acids, ilicic acid methyl ester, costic acid and monoterpenes, geranic acid and (−)-citronellic acid were identified and demonstrated to have significant termite repellent activity (Watanabe et al., 2005a; Watanabe et al., 2005b).

Termite repellent activity has not always been the focus of *Callitris* anti-pest research. Another study of wood oil from *Callitris glaucophylla* focused on mosquitocidal activity using hydrodistilled and solvent extracted oils from wood pulp, which demonstrated significant activity in this regard (Shaalan et al., 2006). In light of another less famous colonial use of the resinous exudate, which was allegedly used to deworm horses following oral administration (Cribb and Cribb, 1981; Low, 1990; Williams, 2011), it is strange that no research has been hitherto focused on vermifugal activity.

In other studies hydrodistillation of sawdust from heartwood of *Callitris glaucophylla* produced guaiol (Doimo et al., 1999) and a significant number of sesquiterpene γ-lactones (Brecknell and Carman, 1979), however solvent extraction produced yet higher concentrations of these larger molecular mass compounds in the subsequent extract (Doimo et al., 1999). Although traces of costols were also characterised in *Callitris glaucophylla* wood oil, these compounds are more common in other *Callitris* species, such as *Callitris intratropica*, which is also known for its azulene rich essential oils; in particular guaiazulene, which is responsible for the oil's blue colour (Doimo, 2001).

Prior to the work of Oprava et al. (2010) no work appears to have been done on the wood pulp oil of *Callitris endlicheri*. Baker and Smith (1910) performed analysis on *Callitris glaucophylla* (as *Callitris glauca* in that study) and proposed that the essential oil may have a similar character to *Callitris endlicheri* (as *Callitris calcarata* in that study) and *Callitris intratropica*. However, in the wisdom of hindsight, subjective observations alone can differentiate these wood pulp essential oils. Such observations include the blue colour in *Callitris intratropica* oils and the yellow to green colour of oils from *Callitris endlicheri* (Oprava et al., 2010). Wood pulp essential oils from *Callitris columellaris*, *Callitris endlicheri*, *Callitris glaucophylla* and *Callitris intratropica* differ in concentrations of guaiol, columellarin, dihydrocollumellarin and guaiazulene (Oprava et al., 2010).

Baker and Smith (1910) were the first to demonstrate the occurrence of guaiol in wood essential oils from these species, apparently crystallising on the cut trunk of species after logging, particularly *Callitris intratropica*. Baker and Smith (1910) were unable to identify another component which they believed had the properties of a phenol. This unknown phenol was tentatively named callitrol in the event that it later proved to be novel. No constituent under the name of callitrol has been subsequently characterised in these wood pulp essential oils (Doimo et al., 1999; Doimo, 2001), nor has any major phenolic constituent been observed corresponding to that suggested by Baker and Smith (1910).

In the earlier study by Baker and Smith (1910) essential oils were also hydrodistilled and characterised from leaves/needles of both species; *Callitris endlicheri* and *Callitris glaucophylla* (as

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Callitris glauca and *Callitris calcarata* respectively). Essential oils from needles of both species were recently more fully characterised by Brophy et al. (2007). A significant difference was noted between these two essential oils, with *Callitris glaucophylla* having significantly higher quantities of monoterpene hydrocarbons, notably α-pinene, myrcene and limonene, with approximately equal quantities of fenchyl- and bornyl acetate (Brophy et al., 2007). The essential oil of needles from *Callitris endlicheri* had notably lower concentrations of these monoterpene hydrocarbons, no fenchyl acetate and significantly higher concentrations of bornyl acetate (Brophy et al., 2007).

Although no antibacterial studies have ever been performed on *Callitris endlicheri* essential oils, preliminary assays, using disc diffusions, were performed on needle essential oils from *Callitris glaucophylla* (Wilkinson and Cavanagh, 2005), demonstrating modest antibacterial activity. Although this study demonstrated possible anti-yeast activity against *Candida albicans*, the absence of any antifungal assays using these respective essential oils is surprising, particularly because *Callitris glaucophylla* timber is also resistant to fungal growth (Brecknell and Carman, 1979; Brophy et al., 2007). We concluded that further, more careful investigation was required, particularly since high antifungal activity of essential oils produced from the wood pulp of New Caledonian *Calltris* species has been observed (Waikedre et al., 2012). Essential oil character from the wood pulp of these species is similar to the Australian *Callitris* species.

With regard to phytochemical studies on *Callitris endlicheri* and *C. glaucophylla*, no characterisation of volatile components has previously been performed on the solvent extracts from needles, or the smoke extracts produced in the laboratory; nor has respective antimicrobial activity been demonstrated. Antifungal activity of pine needle essential oils has not been investigated either. Here then, for the first time, we investigate antimicrobial activity of these extracts and discuss the relevance of this to the volatile compounds subsequently characterised. In this regard, traditional use reports are broadly consistent with antimicrobial activity; however, traditional medicinal applications are likely to be affected by a greater diversity of biological factors, not limited to antimicrobial activities demonstrated here, and this is further discussed later.

2. Materials and methods

2.1. Leaf collection, oil distillation and characterisation

Callitris endlicheri and *Callitris glaucophylla* specimens were collected from various endemic growing locations in New South Wales (NSW) Australia. Corresponding voucher specimens, NJSadgrove81, 83, 242 and 391, were lodged with the N.C.W Beadle Herbarium at the University of New England, Armidale, NSW, Australia. Throughout this manuscript vouchers are cited in shortened form; for example, NJSadgrove81 becomes NS81. Specimens identified as *Callitris glaucophylla* were NS81, NS83 and NS391. A single specimen was subsequently identified as *Callitris endlicheri*, being NS242.

Essential oils were hydrodistilled using approximately 600 g of green needles (fresh, undried). Pine needles were cut into fragments not longer than 5 mm using scissors. Cut needles were then placed into a 5 L round bottomed flask with 2.5 L of deionised distilled water (ddH_20). Needles were heated for 3 h using a 6 L electric mantle and the steam/oil mix was condensed and collected in a 500 mL separating funnel. Oils were separated from the hydrosol and stored away from light at 4 °C until used.

The essential oils were dried to remove hydrosol emulsions using anhydrous Na_2SO_4 powder (0.5 g in 10 mL oil) for more than 24 h prior to GC–MS analysis. Oils were dissolved in dichloromethane (DCM) at a ratio of 1:1000 v/v. Analysis of solvent extracts used 5 mg of crude extract in 2 mL of methanol.

First identifications were performed by comparison of mass spectra with an electronic library database (NIST08) and subsequently confirmed using comparison of temperature programmed retention indices (IUPAC, 1997) with published values. Most discrepancies in identification were resolved by comparison of mass spectra with a second and third library (Joulain and Koenig, 1989; Adams, 2007; NIST, 2011). Quantification was achieved using GC–MS operating software, using data with a minimum peak area of 0.5%.

Analyses were performed using an Agilent Technologies 7890A GC-System coupled with an Agilent 5975C mass selective detector (insert MSD with triple-Axis detector). An autosampler unit (Agilent Technologies 7693–100 positions) was used to perform the 1 μL injections. Separation was accomplished with a HP-5MS Agilent column (30 m × 250 μm × 0.25 μm). Operating conditions were as follows; Injector – split ratio 25:1; Temperature – 250 °C; carrier gas – helium, 1.0 mL/min, constant flow; column temperature – 60 °C (no hold), 5 °C per minute then @ 250 hold 15 min. MS was acquired at −70 eV using a mass scan range of 30–400 *m/z*.

2.2. Antibacterial and anti-Candida activity

Working stocks of all species were maintained on Nutrient Agar (NA) with the exception of *Candida albicans* which required Yeast Extract Peptone Agar (YEPA). All growth media were purchased from Oxoid (Thebarton, South Australia) and prepared according to the manufacturer's instructions.

Minimum inhibitory concentrations (MIC) of the oils and extracts were determined using a micro-titre plate two-fold broth dilution method (CLSI, 2009) with the following modifications. Where essential oil was used, emulsions were prepared by vortexing a measured combination of oil and the appropriate broth with 0.15% w/w agar (Mann and Markham, 1998). Where solvent or smoke extracts were used, milligram quantities were measured then dissolved into acetone or methanol before mixing with agar. Most bacterial species were assayed in Tryptone Soya Broth (TSB) containing 0.15% w/w agar, with the exception of the yeast *Candida albicans* which required YEP broth. Broth dilutions were performed in 96-well plates.

Inoculation was prepared by collecting colonies from fresh working stocks and dispensing into 0.9% w/v NaCl and diluting to match a 0.5 McFarland $BaSO_4$ Turbidity Standard (McFarland, 1907) using a spectrophotometer at 600 nm (or 530 nm for *Candida albicans*). To achieve a final inoculation concentration of 5×10^5 the adjusted saline suspension was diluted into 40 volumes of the appropriate medium and 20 μL was used to inoculate 80 μL of media bringing the total volume to 100 μL and reducing the essential oil concentration to the appropriate target. Following inoculation the 96-well plates were sealed using parafilm and placed into an incubator at 37 °C for approximately 20 h before dispensing 40 μL of sterile 0.2 mg/mL *p*-iodonitrotetrazolium dye and examining for a colour change to red, which indicated organism growth. Positive inhibition controls included tetracycline for bacterial growth and nystatin for *Candida albicans*. Experiments were repeated 3 or more times and the results are reported as the range.

2.3. Fungal sporicidal activity

Fungal sporicidal activity was determined against three species of *Trichophyton* (sourced from the Mycology unit, Women and Children's Hospital, Adelaide) with two strains each; *Trichophyton rubrum* (granular), *Trichophyton rubrum* (not granular), *Trichophyton*

mentagrophytes (sourced from Kangaroo), *Trichophyton mentagrophytes* (sourced from human), *Trichophyton interdigitalis* (Strain 1) and *Trichophyton interdigitalis* (strain 2).

Minimum inhibitory concentrations were determined using mycological peptone prepared with 0.15% w/w agar to achieve a sloppy broth. Inoculation was achieved by dispensing a suspension of fungal spores in 0.9% w/v NaCl solution. Working cultures of *Trichophyton* were propagated on Saboraud Dextrose agar until sporulation occurred. The suspension was prepared by collecting spores from a *Trichophyton* culture, using autoclaved cotton. Cotton was then transferred into 0.9% w/v saline solution and vigorously shaken to release spores into suspension. When used for inoculation the spore suspension was diluted to match a 0.5 McFarland $BaSO_4$ turbidity standard on a spectrophotometer at 600 nm. Diluted suspension was dispensed into the broth or onto the agar at 20% of total volume (20 μL spore solution into 100 μL broth or onto 100 μL agar).

Experiments were maintained at 28 °C for approximately 48 h before inspection for visible signs of growth. Signs of growth were conceded if the broth had become unclear or translucent, or if the agar had a visible *Trichophyton* formation growing on the surface of it. Experiments were repeated 3 or more times and the results are reported as the range.

2.4. TLC-bioautography

Extracts were dissolved into methanol at a ratio of 1:1, then applied in a volume of 2 μL onto aluminium backed silica gel TLC plates (Merck kieselgel 60 F254). The solvent system was hexane: ethyl acetate, 25:75 by volume. The solvent chamber was allowed to equilibrate for 10 min before plates were inserted. Following separation the plates were air dried for 1 h to allow solvent to completely evaporate prior to overlay with the target organism in agar. Duplicate TLC plates were prepared and run in the same chamber; one for staining and the other for bioautographic overlay.

The bioautography was performed in sterile conditions with *Staphylococcus aureus* (ATCC 29213) as the target organism overlay in agar (1% w/w). Nutrient agar was maintained as a liquid after autoclaving by placing into a rocking water bath at 40 °C. The agar was seeded with 100 mg/L *p*-iodonitrotetrazolium dye then inoculated with the test organism after matching to a 0.5 McFarland $BaSO_4$ turbidity standard in saline. Inoculations used saline at approximately the same temperature as the agar (otherwise the organism was not viable). Seeded agar was set over the TLC plate covering a thickness of not more than 3 mm and remained for 20 h at 38 °C incubation before inspection. TLC separated components of solvent extracts with antibacterial activity appeared as clear zones against a red background.

2.5. Solvent and smoke extracts

To produce solvent extracts, leaves were finely chopped and soaked in respective solvent (acetone or methanol). Approximately 50 g of plant matter was soaked in 80 mL solvent for approx. 24 h in an incubator at 40 °C. Solvent was separated through a strainer and allowed to completely evaporate under a fume hood, then stored at −20 °C until used. Additionally, pine needles from NS391 (*Callitris glaucophylla*) were boiled for several hours and the subsequent decoction was partitioned into *n*-butanol. The extract was then evaporated and stored in the same manner.

Smoke extracts were produced using two methods. In the first method, depicted in Fig. 1 (Method A), three *Callitris* specimens were used (*Callitris glaucophylla* – NS83, *Callitris endlicheri* – NS242 and *Callitris glaucophylla* – NS391). This method involved placing finely chopped needles into a round bottom flask. The flask was then heated over a mantle until smoke was produced (approx. 20 min). During this 20 min period, a vacuum was applied to draw

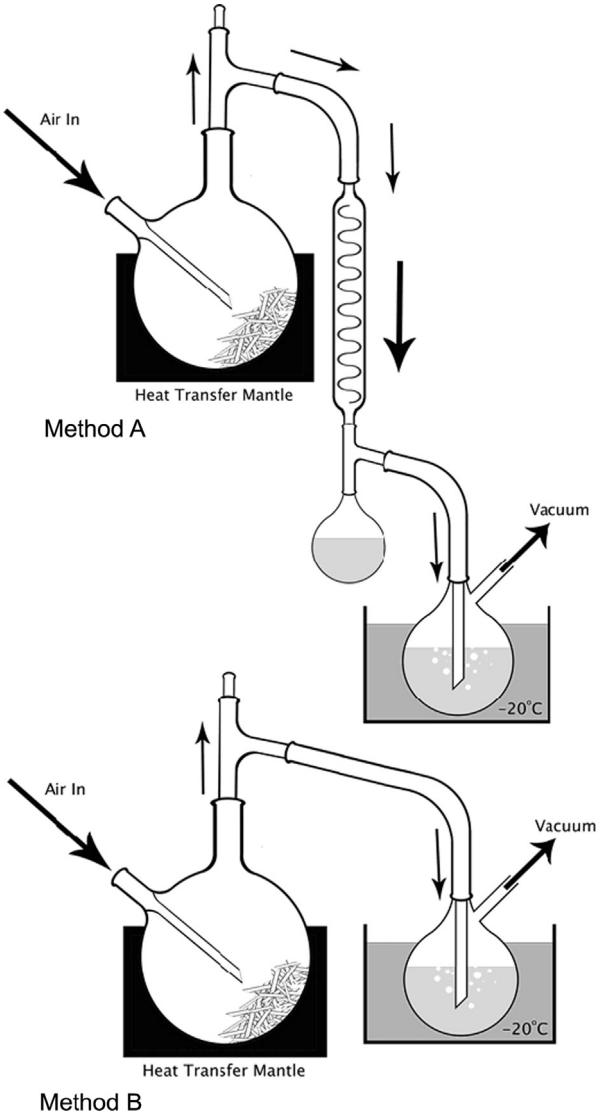

Fig. 1. Methods for smoke extraction of *Callitris* needles.

air into the round bottom flask, delivering directly to the needles. Smoke was then directed through a condenser and afterwards bubbled through solvent (methanol/acetone 1:1) at −20 °C. A flask was included below the condenser to capture the condensed liquid material fraction. Both condensate and bubbled fractions were included in antimicrobial testing.

The second method, also depicted in Fig. 1 (Method B), was used for only one specimen (NS391). Needles were placed into a round bottom flask, heated and vacuumed in the same manner as the former smoking method described above. However, the condenser was excluded and the solvent used was deionised distilled H_2O. The subsequent water extract was partitioned into chloroform, and then afterwards the same material was differentially partitioned into *n*-butanol. Organic extracts were evaporated under a fume hood and then stored at −20 °C for later use in antimicrobial testing.

3. Results

Essential oils from both *Callitris* species conformed in chemical character to previous reported characterisations (Brophy et al., 2007), with major components for *Callitris glaucophylla* (NS81, NS83 and NS391) being α-pinene, limonene, fenchyl- and bornyl

acetate (Table 1). Major components of essential oils from *Callitris endlicheri* (NS242) were bornyl acetate, nerolic acid, nerol acetate and geranyl acetate (Table 1).

Characterisation of the volatile fraction of methanol extracts from two specimens of *Callitris glaucophylla* (NS83 and NS391) and one specimen of *Callitris endlicheri* (NS242) demonstrated the presence of most components also found in the essential oils (Table 2), but with additional sesqui- and diterpenes not detected in the essential oils, most of which could not be identified. We did however identify the abietane diterpenes ferruginol, pisiferal and pisiferol in *Callitris glaucophylla* specimens (NS83 and NS391). These comprised the major components of the larger molecular mass fraction of volatile constituents. In the extract from *Callitris endlicheri* (NS242) these components were not detected, but an unknown diterpene, representative of 25% of the volatile fraction (Unknown I) was present. In both species, catechol was also detected (Table 2).

As expected, in smoke extracts a large number of volatile compounds were reported that also occur in coal and wood tar distillation (Lai and Song, 1995; Lee et al., 2005; Zhang et al., 1992), cigarette or wood smoke (Table 3); collectively these are of phenolic or furanic character. Commonly known phenolic major constituents characterised are creosol (4-methylguaicol), acetophenone and catechol, the latter which was also present in the solvent extracts of the fresh needles at an appreciable concentration. Additionally, the abietane diterpene pisiferal was also found in the smoke extract made of the *Callitris glaucophylla* specimen (NS391) at approx. 1–2% (Table 3). Differential solvent partitioning of the smoke water extract, using chloroform first followed by *n*-butanol, produced relatively similar chemical profiles with regard to minor components. However, major components, such as phenol, creosol and other cresols, were all extracted in significantly higher concentrations using *n*-butanol. Additionally, catechol, 3-methylcatechol and homocatechol, were only present in the *n*-butanol partition, also present at substantially high concentrations, most notably catechol, at approx. 18%.

Mass spectral data of significant unknown compounds from Tables 2 and 3 are summarised in Table 4. In Tables 5 and 6 the mean inhibitory concentrations (MIC) are presented, respectively showing antifungal and antibacterial activity. Solvent extracts of both *Callitris* species exhibited no inhibition against fungal spore growth from *Trichophyton* species (Table 5), at the starting concentrations used (11–12 mg/mL). Conversely, solvent extracts produced selective inhibitions against bacterial species (Table 6), particularly *B. subtilis*, *S. aureus* and *S. pneumoniae*, with MIC concentrations as low as 100 μg/mL for *B. subtilis* and *S. aureus*, from the whole acetone extract of NS83 (*Callitris glaucophylla*); and others ranging from 200 to 400 μg/mL, except solvent extracts from NS391, which were significantly less active than the other two specimens.

Antimicrobial activity (MIC) of needle essential oils was modest (Tables 5 and 6), with again significantly less activity demonstrated by NS391 (*Callitris glaucophylla*). With regard to antifungal *Trichophyton* spore growth inhibition, significantly greater activity was observed using essential oils from NS242 (*Callitris endlicheri*), with MIC concentrations averaging around 7 mg/mL, but as low as 3.5 mg/mL. With regard to smoke extracts, using Method A in Fig. 1, which subsequently produced two extracts, one bubbled into solvent (i.e., 83 bub) and one as the condensate (i.e., 83 cond), MICs were again significantly higher against the species, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*, with the greatest activity produced from bubbled extracts. This is also consistent with activity from solvent extracts.

In some cases, such as with *P. aeruginosa* and *S. pneumoniae*, greater MICs were produced using the smoked condensate fraction (Table 6). However, with regard to antifungal spore inhibition, no significant difference was noted between these two fractions (Table 5). Be this as it may, antifungal activity was uniformly high, with MIC concentrations ranging from 0.7 to 2.2 mg/mL. Using the second method for producing smoke extracts (Fig. 1 – Method B), the fraction partitioned into *n*-butanol after the earlier wash with chloroform, produced the greatest antifungal activity, with concentrations averaging at 0.7 mg/mL. Antibacterial MICs were also high, but with the greater activity produced from the chloroform partition, with concentrations ranging from 0.3 to 1.4 mg/mL.

Lastly, the *n*-butanol partition, produced from a decoction of boiled pine needles from NS391 (*Callitris glaucophylla*) produced modest antifungal MICs (11.3 mg/mL) and modest antimicrobial MICs, with the greatest activity as low as 0.9 mg/mL.

Table 1
Essential oils characterised using GC–MS with calculated arithmetic indices (AI) shown alongside published values (Pub. AI) (Adams, 2007).

	AI	Pub. AI	NS81	NS83	NS242	NS391
α-Tricyclene	925	923	1.3	0.6	0.2	1.5
α-Thujene	928	927	–	–	–	0.2
α-Pinene	936	939	16.7	17.4	0.7	22.8
Camphene	951	954	2.9	2.7	0.5	3
β-Pinene	980	979	–	0.4	–	0.4
Myrcene	992	988	6.8	9.2	1.3	6.9
α-Terpinene	1018	1017	–	–	–	0.2
p-Cymene	1027	1023	–	0.5	0.4	0.5
Limonene	1031	1029	18.2	22.2	4.2	26.2
γ-Terpinene	1060	1059	–	0.3	–	0.5
Terpinolene	1091	1086	1.7	–	–	1.2
Linalool	1101	1100	–	–	0.4	–
Fenchol	1117	1118	–	0.2	–	–
Camphor	1148	1150	1.5	3.2	1.4	2.1
Neo-isopulegol	1154	1144	–	–	3.4	–
Isopulegol	1159	1145	–	–	0.3	–
Borneol	1169	1165	1.3	0.3	1.1	0.2
Terpinen-4-ol	1180	1177	–	0.3	0.5	0.2
Terpineol	1193	1188	–	0.2	0.6	–
Fenchyl acetate	1223	1220	17.5	19.7	–	11.7
Citronellol	1229	1223	–	–	3.1	–
Geraniol	1256	1252	–	–	0.7	–
Neo-isopulegyl acetate	1278	1274	–	–	0.8	–
Bornyl acetate	1292	1287	28.1	16.1	47.4	16.1
Nerolic acid	1355	1351	–	1.9	7.7	1.8
α-Terpineol acetate	1357	1350	2.5	–	–	–
Nerol acetate	1367	1359	–	–	9.1	–
Geranyl acetate	1387	1379	–	–	14.5	–
Caryophylene	1425	1423	–	1.5	–	2.1
Humulene	1459	1456	1.6	0.3	–	0.3
Caryophyllene oxide	1589	1589	–	–	–	0.5
Spathulenol	1584	1577	–	–	0.6	–

Numerical values correspond to voucher references, i.e., NS83 refers to voucher NJSadgrove83. Both NS83 and NS391 are from *Callitris glaucophylla* and NS242 is from *Callitris endlicheri*.

4. Discussion

4.1. Phytochemistry and bioactivity

The occurrence of highly active catechol, phenol and creosol in smoke extracts may shed light on traditional use of *Callitris* species in Australian Aboriginal medicinal practice. In western medicine phenol was one of the first effective antiseptic agents and it has been widely employed as a wound dressing or in food preservation (Hugo, 1978). Creosol is one of the major constituents of coal and wood tar distillate producing creosote. Both coal tar and beechwood distillate (wood tar distillate from members of the Fagus genus; beech trees) have been removed from Western medicinal practices because of potential carcinogenic activity (Lee et al., 2005);

Table 2
GC–MS analysis of the volatile fraction collected in solvent extracts produced using methanol (MeOH). Arithmetic indices (AI) were calculated and are compared here with published values (Pub. AI). The Arithmetic indices references (AI Ref.) are as follows; (a) Adams (2007), (b) Harrison and Priest (2009), (c) Adams (1999), (d) Kosyukova and Khorguani (1989) (e) da Silva et al. (1999) (f) Asuming et al. (2005) and (g) Kukic et al. (2006).

	AI	Pub. AI	AI Ref.	NS391 MeOH	NS83 MeOH	NS242 MeOH
α-Pinene	934	939	a	1.8	–	–
Mycene	991	988	a	0.8	–	–
p-Cymene	1025	1023	a	–	–	1.1
Limonene	1030	1029	a	3.2	–	–
1,8-Cineol	1033	1026	a	–	–	1.1
Unknown amine 1	1078	–	–	–	1.3	–
4 H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1143	1153	b	–	1.8	2.3
Camphor	1147	1150	a	2.0	1.4	0.8
Borneol	1168	1165	a	–	–	0.5
Terpineol	1193	1188	a	–	–	0.5
Catechol	1199	1197	e	0.8	3.1	3.5
Fenchyl acetate	1222	1220	a	6.7	4.2	–
5-Hydroxymethylfurfural	1228	1238	b	–	1.7	4.0
Geraniol	1256	1252	a	–	–	0.7
Bornyl acetate	1289	1287	a	9.9	5.0	15.3
p-vinyl-Guaiacol	1315	1309	a	–	2.0	–
exo-2-Hydroxycineole acetate	1345	1345	a	0.4	–	–
α-Terpineol acetate	1352	1350	a	1.4	0.9	–
(*Z*)-α-Damascone	1354	1355	a	–	–	3.6
Nerol acetate	1366	1351	a	–	–	2.9
Geranyl acetate	1385	1379	a	–	–	5.1
Unknown amine 2	1398	–	–	–	1.5	–
Caryophyllene	1425	1423	a	9.6	6.1	–
Aromandendrene	1444	1439	a	0.5		–
Humulene	1459	1456	a	1.2	0.9	–
β-Thujaplicin	1481	1475	a	–	1.7	–
β-Selinene	1491	1498	a	0.5	–	–
3-Hydroxybenzoic acid	1494	–	a	–	–	3.5
Unknown M	1534	–	–	–	–	5.6
Spathulenol	1584	1577	a	–	–	1.2
Caryophyllene oxide	1589	1589	a	1.8	1.4	–
Ledol	1610	1602	a	–	–	3.0
Unknown G	1732	–	–	–	–	2.7
n-Hexadecanoic acid	1961	1959	a	–	4.3	–
Unknown furan	2090	–	–	–	1.8	–
Phytol	2113	2111	g	1.0	1.5	1.8
α-Linoleic acid	2139	2143	f	0.3	3.5	1.1
Cryptopinone	2192	2154	d	0.6	–	–
Decyl anthranilate	2232	2241	a	–	–	1.6
Unknown H	2283	–	–	–	–	1.2
Unknown I	2321	–	–	–	–	25.5
Ferruginol	2333	2325	c	3.8	4.4	–
Uknown A	2374	–	–	9.0	4.6	–
Unknown B	2409	–	–	2.0	5.3	–
Unknown J	2415	–	–	–	–	3.8
Unknown C	2478	–	–	4.7	3.9	–
Pisiferal	2540	–	–	25.1	30.2	–
Pisiferol	2580	–	–	1.1	7.2	–
Unknown N	2660	–	–	2.1	–	–

Numerical values correspond to voucher references, i.e., NS83 refers to voucher NJSadgrove83. Both NS83 and NS391 are from *Callitris glaucophylla* and NS242 is from *Callitris endlicheri*.

however, beechwood creosote is still held in high regard in Japanese medicinal pharmacopoeia. Approximately 21% of beechwood creosote is creosol, 25% is guaicol and the remainder is made up of low molecular mass phenolics, such as *p*- *m*- or *o*-cresol (Lee et al., 2005). Creosol is also commonly known, and referred to in other studies, by the names p-methylguaicol, 4-methylguaicol, 2-methoxy-4-methylphenol or homoguaicol.

Interestingly, both Japanese and Korean herbalists mix beechwood creosote with other herbs for the treatment of intestinal troubles, such as poisoning and diarrhoea. Studies have demonstrated that this antidiarrheal activity may in part be due to antisecretory activity, also demonstrated by loperamide, a commercial antidiarrheal drug (Lee et al., 2005). Beechwood creosote is relatively colourless to translucent yellow. The *n*-butanol extract of NS391 smoked in this study was a translucent orange colour.

Catechol is a naturally occurring antibiotic (Kocacalıskan et al., 2006). Unlike creosol, catechol is not only produced by heating or smoking the leaves, but is also often present in the source material, as was demonstrated by the character of our solvent extracts and by analysis performed in other studies focused on the phytochemistry of pine needle litter at the base of various ash and pine species (Kuiters and Sarink, 1986). With regard to the occurrence of catechol in leaf litter, Olsen et al. (1971) demonstrated that significant quantities of catechol and phenolic acid in leaf litter from *Populus tremula* had a strong selective inhibitory effect on corrhizal fungi but not on leaf decomposers. Thus, the significantly higher antifungal activity produced by the *n*-butanol partition of the smoke extracts in this study may be related to the occurrence of catechol at approx. 18%. Interestingly, the observation of significant inhibition of the soil microbe *Bacillus subtilis*,

Table 3
Smoke extracts analysed using GC–MS. Arithmetic indices (AI) were calculated and compared with published values (Pub. AI). The source of the published AI (AI Ref.) is as follows; (a) Adams (2007), (b) Wanakhachornkrai and Lertsiri (2003) (c) Pino et al. (2001), (d) Mateo et al. (1997), (e) Re-Poppi and Santiago (2002), (f) Chassagne et al. (1999), (g) Song et al. (2003), (h) Harrison and Priest (2009), (i) Xu et al. (2003), (j) da Silva et al. (1999), (k) Guyot et al. (1998), and (l) Radulovic et al. (2009).

	AI	Pub. AI	AI Ref.	NS242-$\pi\rho$ $CHCl_3$	NS391-$\pi\rho$ BuOH	NS391-$\pi\rho$ $CHCl_3$
Pyridine	750	747	a	–	–	0.3
Cyclopentanone	795	797	a	–	–	0.5
Furfural	825	828	a	1.9	–	–
2-Cyclopenten-1-one	833	835	i	–	–	2.3
2-Furanmethanol	853	875	d	–	1.0	1.0
1-Acetyloxy-2-propanone	866	869	h	–	–	1.1
o-Xylene	869	863	c	–	–	0.7
2-Methyl-2-cyclopenten-1-one	908	907	h	–	–	1.6
2*E*,4*E*-Hexadienal	908	909	a	0.7	–	–
2-Acetylfuran	912	909	a	0.5	–	2.0
5-Methylfurfural	962	957	a	2.2	–	1.1
3-Methyl-2-cyclopenten-1-one	965	973	d	–	–	2.2
Phenol	981	989	b	4.9	16.5	8.7
p-Cymene	1026	1023	a	0.6	–	0.6
3-Methyl-1,2-cyclopentanedione	1026	1017	a	–	1.1	1.0
Limonene	1030	1029	a	1.0	–	4.0
2,3-Dimethyl-2-cyclopenten-1-one	1040	1040	h	–	–	1.1
o-Cresol	1052	1050	a	2.4	3.0	3.4
Acetophenone	1067	1059	a	0.8	–	0.4
p-Cresol	1072	1071	a	5.3	11.0	8.3
o-Guaiacol	1091	1087	a	4.9	3.3	7.5
3-Pyridinol	1103	–	–	–	1.3	–
allo-Ocimene	1130	1128	a	0.4	–	–
o-Ethylphenol	1137	1137	e	0.6	–	0.4
3,5-Dimethylphenol	1148	1145	k	–	–	2.6
2,5-Dimethylphenol	1147	1148	e	–	1.5	0.8
Camphor	1148	1150	a	3.2	–	–
4-Ethylphenol	1165	1163	f	1.8	4.0	4.0
3,5-Dimethylphenol	1169	1170	l	–	1.3	1.8
Naphthalene	1186	1178	a	0.8	–	–
Creosol	1194	1188	a	3.5	1.0	3.0
Catechol	1197	1197	j	–	18.4	–
Dihydrobenzofuran	1218	1211	h	–	–	2.0
Fenchyl acetate	1222	1220	a	–	–	1.6
3-Methylcatechol	1260	1254	a	–	3.1	–
Unknown L	1262	–	–	–	–	1.1
Unknown K	1266	–	–	–	–	1.2
Hydroquinone	1272	–	–	–	3.8	–
p-Ethylguaiacol	1280	1285	e	2.4	1.1	2.2
Bornyl acetate	1288	1287	a	15.2	–	1.9
Homocatechol	1289	1293	e	–	7.7	
Indole	1295	–	–	–	–	1.1
Benzocycloheptatriene	1296	–	a	1.5	–	–
Tridecane	1300	1300	a	0.4	–	–
p-vinylguaiacol,	1315	1309	a	0.9	–	1.2
o-Isopropyl-α-methylstyrene	1328	–	a	1.1	–	–
3-Hydroxybenzenemethanol	1343	1339	a	–	3.1	–
Nerolic acid	1354	1351	a	4.4	–	–
Syringol	1353	1355	e	–	–	1.3
4-Ethylcatechol	1383	1392	h	–	3.2	–
(*Z*)-1-Tetradecene	1392	1388	a	0.7	–	–
Tetradecane	1400	1400	a	0.4	–	–
2,7-Dimethylnaphthalene	1406	1409	g	0.5	–	–
1,4-Dimethylnaphthalene	1424	1424	g	2.8	–	–
Caryophyllene	1424	1423	a	–		1.5
m-Carbomethoxyphenol	1425	1429	a	–	1.2	–
Cyclamen aldehyde	1463	1459	a	6.0	–	–
1,4-Dihydronaphthalene 2,5,8-trimethyl-	1547	–	a	1.6	–	–
1,6,7-Trimethylnaphthalene,	1589	1572	a	3.6	–	–
1,2,3,4-Tetrahydro-5,6,7,8-tetramethylnaphthalene	1617	–	a	0.4	–	–
Methyl hexadecanoate	1935	1921	a	0.7	–	–
Ambrettolid	1939	1929	a	1.4	–	1.2
Unknown F	1957	–	–	–	–	1.9
1,7-Dimethylphenanthrene	2047	–	–	–	–	0.6
Unknown D	2194	–	–	–	–	1.1
Unknown D – 1st isomer	2269	–	–	–	–	1.0
Unknown D – 2nd isomer	2292	–	–	–	–	2.6
Unknown D – 3rd isomer	2324	–	–	–	–	2.3
Unknwon E	2372	–	–	–	–	1.7

Table 3 (*continued*)

	AI	Pub. AI	AI Ref.	NS242-πρ $CHCl_3$	NS391-πρ BuOH	NS391-πρ $CHCl_3$
Unknown D – 4th isomer	2437	–	–	–	–	2.6
Pisiferal	2534	–	–	–	–	1.6

Numerical values correspond to voucher references, ie., NS391 refers to voucher NJSadgrove391. NS391 is from *Callitris glaucophylla* and 242 is from *Callitris endlicheri*. NS391-πρ $CHCl_3$ uses the greek letters for pi (π) and ro (ρ) to refer to pyrolysed then bubbled through H_2O using the second method for smoke extracts (Fig. 1: Method B) which were then differentially partitioned firstly into chloroform ($CHCl_3$) then *n*-butanol (BuOH).

Table 4
Mass spectral data of unknown compounds in Tables 2 and 3.

Compound	AI	*m/z*
Unknown L	1262	152 (11), 145 (17), **140 (100),** 137 (24), 125 (72), 107 (26), 97 (56), 78 (44), 69 (18), 51 (27), 39 (25)
Unknown K	1266	166 (4), 150 (78), 144 (32), 129 (54), 121 (54), 107 (35), **91 (100),** 77 (82), 65 (57), 55 (30), 39 (43)
Unknown M	1534	222 (3), 204 (2), 189 (7), 161 (12), 149 (23), **126 (100),** 121 (22), 108 (98), 93 (30), 81 (13), 67 (26), 55 (23), 43 (46)
Unknown G	1732	214 (2), 188 (1), 154 (2), 136 (17), **121 (100),** 107 (16), 93 (27), 87 (28), 81 (24), 73 (23), 67 (14), 55 (17), 43 (79)
Unknown F	1957	256 (68), **241 (100),** 227 (33), 213 (46), 199 (7), 185 (17), 173 (28), 159 (28), 145 (35), 131 (40), 119 (35), 108 (56), 93 (69), 79 (41), 67 (21), 55 (21), 41 (23)
Unknown D	2194	286 (78), **271 (100),** 243 (11), 187 (9), 161 (15), 149 (13), 115 (11), 91 (9), 69 (10), 55 (9), 41 (9)
Unknown D – 2nd	2269	272 (100), **257 (100),** 229 (18), 202 (7), 187 (16), 173 (25), 159 (17), 147 (32), 133 (18), 115 (11), 91 (9), 69 (11), 55 (9), 41 (11)
Unknown H	2283	358 (6), 316 (33), 287 (14), 171 (20), 257 (20), 241 (22), 215 (21), 201 (10), 189 (29), 175 (22), 163 (25), 147 (32), **137 (100),** 121 (78), 107 (64), 93 (74), 79 (66)
Unknown D – 3rd	2292	272 (94), **257 (100),** 229 (18), 187 (13), 173 (16), 159 (9), 147 (28), 135 (22), 115 (11), 91 (7), 69 (11), 55 (8), 41 (10)
Unknown I	2321	302 (4), 287 (27), 257 (25), 241 (27), 219 (9), 187 (17), 173 (16), 161 (18), 147 (23), 133 (39), **121 (100),** 107 (47), 93 (79), 67 (36), 55 (35), 41 (46)
Unknown D – 4th	2324	270 (29), **255 (100),** 211 (4), 165 (7), 120 (5), 91 (4), 41 (4)
Unknown E	2372	**270 (100),** 255 (61), 227 (21), 114 (36), 199 (50), 171 (52), 153 (13), 128 (12), 91 (11), 41 (8)
Unknown A	2374	**270 (100),** 255 (61), 227 (20), 215 (41), 199 (50), 171 (53), 153 (11) 128 (13)
Unknown B	2409	344 (32), 302 (24), 287 (26), 257 (18), 241 (23), 187 (21), **137 (100),** 121 (55), 105 (48), 91 (47), 79 (45), 55 (32), 41 (30)
Unknown J	2415	344 (34), 271 (8), 189 (11), 157 (7), **137 (100),** 124 (15), 107 (11), 91 (11), 69 (15), 55 (13), 43 (11)
Unknown D – 5th	2437	268 (54), **253 (100),** 211 (22), 165 (11), 119 (6)
Unknown C	2478	302 (7), **176 (100),** 161 (24), 147 (7), 91 (4), 55 (4)
Unknown N	2660	358 (24), 330 (5), 302 (6), 271 (13), 241 (7) 189 (14), **137 (100),** 121 (13), 91 (16), 55 (14)

Table 5
Mean inhibitory concentration (MIC) for sporicidal antifungal activity in mg/mL. Nystatin was in μg/mL. Organisms were from the *Trichophyon* genus, *Trichophyon rubrum* granular strain and non-granular strain, *Trichophyon interdigitalis* strain 1 and 2, and *Trichophyon mentagrophytes* isolated from a human and kangaroo.

	T. r gran	T. r non-gran	T. in 1	T. in 2	T. m hum	T. m kang
83 bub	1.5	1.5	1.5	0.7	0.7	0.7
83 cond	1.5	1.5	3	1.5	1.5	1.5
242 bub	1.5	1.5	1.5	0.8	1.5	1.5
242 cond	2.2	1.1	1.1	1.1	1.1	2.2
83 EO	>	10.5	21	10.5	>	10.5
242 EO	3.5–10.5	7	7–10.5	5.6–7	3.5–10.5	7–10.5
391 EO	>	>	>	>	>	>
83 Acetone	>	>	>	>	>	>
83 MeOH	>	>	>	>	>	>
242 Acetone	>	>	>	>	>	>
242 MeOH	>	>	>	>	>	>
391 Acetone	>	>	>	>	>	>
391 MeOH	>	>	>	>	>	>
391 Boil BuOH	11.3	11.3	11.3	11.3	11.3	11.3
391-πρ H_2O $CHCl_3$	0.9	1.8	0.9	0.9	0.9	0.9
391-πρ H_2O BuOH	0.7	1.4	0.7	0.7	0.7	0.7
Nystatin	2 μg/mL	8 μg/mL	4 μg/mL	4 μg/mL	8 μg/mL	4 μg/mL

Numerical values correspond to voucher references, i.e., 83 refers to voucher NJSadgrove83. Both 83 and 391 are from *Callitris glaucophylla* and 242 is from *Callitris endlicheri*. Suffixes are as follows: 'bub' refers to the first method for producing smoke extracts (Fig. 1: Method A) involving the bubbling of smoke/steam through a solvent, 'cond' refers to the condensate collected from the condenser. EO refers to the essential oil. Acetone and MeOH (methanol) refer to the solvents used to produce solvent extracts. 391-πρ H_2O uses the greek letters for pi (π) and ro (ρ) to refer to pyrolysed then bubbled through H_2O using the second method for smoke extracts (Fig. 1: Method B) which were then differentially partitioned firstly into chloroform ($CHCl_3$) then *n*-butanol (BuOH).

using our solvent extracts, may also be of relevance in considering this ecological reciprocal inhibition.

Other compounds detected in both the solvent and smoke extracts in this study were the abietane diterpenes, particularly pisiferal. Pisiferal was first characterised from the Japanese species, *Chamaecyparis pisifera* (Cupressaceae) (Hasegawa et al., 1985; Xiao et al., 2001; Yatagai and Takahashi, 1980). Thus, these diterpenes observed by us have already been noted in other Cupressaceae, including pisiferol and ferruginol.

With regard to these three diterpenes and their congeners, a significant number of bioactive effects have been demonstrated *in vitro*. Firstly, significant antibacterial activity of pisiferal and pisiferol

Table 6
Mean inhibitory concentrations (MIC) from antimicrobial activity of extracts in mg/mL. The control was either tetracycline for bacterial species of nystatin for yeast (*Candida albicans*) in μg/mL. Bacterial species are *Pseudomonas aeruginosa, Klebsiella aerogenes, Escherichea coli, Bacillus subtilis, Salmonella typhimirium, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae* and *Candida albicans*.

	P. aeru	***K.aero***	***E. coli***	***B. sub***	***S. typh***	***S. epi***	***S. aur***	***S. pneu***	*Callitris* ***alb***
83 bub	2.6	1.5	10.4	0.3–0.4	3–5.2	3–5.2	0.3–0.4	3	0.8–2.6
83 cond	1.5–2.6	NA	5.2	5.2	10.4	5.2	2.6	0.7	5.2
242 bub	2.6	3	10.4	0.2–0.3	>	5.2	0.2–0.7	1.1	0.8–2.6
242 cond	1.5–2.6	NA	10.4	3.6	>	5.2	2.6	0.4	2.6
391 Bub	2.5–2.6	2.5	10.4	0.3–1	5–10.4	5–10.4	0.3–1.6	NA	1–2.2
391 Cond	1.5–2.2	2.5	5.2–10	2.5–5.2	5	5–5.2	2.5–5.2	NA	2.5–10.4
83 EO	2.1	8.34	>	0.3	>	>	0.5	NA	NA
242 EO	2	5.1–8.14	>	0.3–1.3	2.6	10.3	1–1.3	NA	10.3
391 EO	10.5	10.5	10.5	5.3	10.5	>	5.3	NA	>
391 Acetone	5.5	2.8	1.4	2.8	5.5	1.4	2.8	NA	>
391 MeOH	>	>	>	0.3	>	>	5.3	NA	10.5
391 Boil BuOH	>	3.4	7	3.5	0.9	7	0.9	NA	3.5
391-πρ H_2O $CHCl_3$	0.3	1.4	0.7	0.3	0.7	1.4	0.3	NA	0.3
391-πρ H_2O BuOH	1	2	2	0.25	1	4	1	NA	1
83 Acetone	5.5	5.5	2.6	0.1	5.5	5.5	0.1	0.4	>
83 MeOH	>	NA	>	0.3	>	>	0.3	0.4	>
242 Acetone	0.7	2.8	>	0.2	5.5	5.5	0.2	0.4	>
242 MeOH	>	NA	>	0.3	>	>	0.2	0.4	>
Control	2–4	0.1–0.13	0.5	0.25–0.5	0.1–0.13	0.25–0.3	0.1–0.13	0.5	1.5

Numerical values correspond to voucher references, ie., 83 refers to voucher NJSadgrove83. Both 83 and 391 are from *Callitris glaucophylla* and 242 is from *Callitris endlicheri*. Suffixes are as follows; 'bub' refers to the first method for producing smoke extracts (Fig. 1: Method A) involving the bubbling of smoke/steam through a solvent, 'cond' refers to the condensate collected from the condenser. EO refers to the essential oil. Acetone and MeOH (methanol) refer to the solvents used to produce solvent extracts. 391-πρ H_2O uses the greek letters for pi (π) and ro (ρ) to refer to pyrolysed then bubbled through H_2O using the second method for smoke extracts (Fig. 1: Method B) which were then differentially partitioned firstly into chloroform ($CHCl_3$) then *n*-butanol (BuOH).

was demonstrated against *Staphylococcus aureus* and *Bacillus subtilis* (Xiao et al., 2001). Although this is interesting with regard to results produced in this study, solvent extracts from NS242 (*Callitris endlicheri*) also produced significant inhibitory effects against these two bacterial species, but these abietane diterpenes were not characterised in this specimen. Bioautography demonstrated that the main inhibitory compound is significantly more polar than would be expected from these respective diterpenes and its occurrence is unconfirmed in the smoke extract. The expected polarity of this antimicrobial compound indicates that it is hydrophilic, which implicates its occurrence in traditional aqueous extracts produced by Australian Aboriginal people.

Because solvent extracts from NS391 (*Callitris glaucophylla*) were substantially less active against *Staphylococcus aureus* and *Bacillus subtilis*, this unknown compound may either be absent or substantially less concentrated in that specimen, which indicates potential chemovariation in this regard. Such possible chemovariation will be clarified in subsequent, more elaborate studies. We will also seek to elucidate the structure of this compound and its differential distribution within the genus.

A number of other inhibition zones were observed in the bioautographic study migrating further toward the solvent front. In terms of their relative hydrophobicity then, these may be the respective abietane diterpenes. In previous studies, in addition to antimicrobial activity, these compounds also demonstrated in vitro activity consistent with an anti-inflammatory response. Pisiferal showed inhibitory activity against the release of β-glucuronidase in rat polymorphonuclear leucocytes induced by platelet-activating factor (Lin et al., 2010). Interestingly, Japanese scientists have patented dentrifices containing pisiferal, pisiferol and pisiferic acid (JP 63230621 – Dentifrices containing pisiferic acid and its derivatives). It is tempting to consider this modern proposed use in the context of the earlier Australian colonial uses of resinous exudate from *Callitris glaucophylla* as a filling for teeth.

Another patent describes using pisiferal for controlling acne and dandruff (JP 63119410 and JP 07084371 – Topical formulations containing pisiferic acid derivatives controlling acne and dandruff). Yet another describes using pisiferal, pisiferal and pisiferic acid for skin disorders (JP 62270547 – Manufacture of substances containing pisiferic acid for treatment of skin disorders). Again, it is tempting to consider this modern proposed use in the context of traditional Aboriginal Australian topical uses of lipophilic extracts of pine needles from *Callitris glaucophylla*.

Other studies have demonstrated antimite, antifly, antioxidative and antibacterial activity from pisiferic acid and its congeners (Yatagai and Nakatani, 1994; Yatagai, 1997). Although pisiferic acid was not specifically characterised in any of our extracts herein investigated, further studies will seek clarification in the light of traditional use reports of *Callitris glaucophylla* extracts for the treatment of scabies. Again, although sesquiterpene γ-lactones, with significant termite repellent activity, were previously characterised from wood pulp essential oil from *Callitris* species (Watanabe et al., 2005a; Watanabe et al., 2005b), such compounds were not identified in any of our extracts. This may indicate their exclusive presence in the woody tissue of the plant and not the pine needles.

Two of these γ-lactones, columellarin, dihydrocolumellarin, have *in vitro* activity consistent with an anti-inflammatory response, as indicated by enhancement of granulocyte and monocyte activity in a phagocytosis assay (Oprava et al., 2010). Again though, these compounds were not found by us in the pine needles.

Another of the abietane dieterpenes found in solvent extracts from *Callitris glaucophylla* specimens in this study, also previously characterised in Cupressaceae, was ferruginol, which also has a significant number of previously described bioactivities. Ferruginol has been associated with gastroprotective effects (Areche et al., 2008), antifungal, anti-tumor, anti-maliaral, cardio-protective, anti-plasmodial activity and others (Wei et al., 2009). Additionally, ferruginol derivatives have been demonstrated to have selective cytotoxic activity against gastric cancer cell lines (Espinoza et al., 2008).

With regard to antifungal activity and its relationship to the fungal resistance of *Callitris* timbers, only one of our essential oils screened here was able to produce moderate antifungal activity (NS242, *Callitris endlicheri*). The main difference of this active essential oil was the occurrence of geranyl acetate, nerol acetate and nerolic acid. A previous study demonstrated good fungicidal activity of geranyl acetate against *Candida albicans* (Zore et al., 2011). Having said this, antifungal activity of *Callitris* timber is more

likely to be related to the respective γ-lactones produced from the timber, already shown to be associated with good antifungal activity in a New Caledonian study (Waikedre et al., 2012).

4.2. Traditional Aboriginal Australian medicinal use

As far as we can tell, no recorded traditional Aboriginal uses are consistent with the most common colonial uses previously described. However, two lesser known colonial uses more closely resemble usage modalities undertaken by Australian Aboriginal people. The first was by Maiden, who stated that 'It is one of the most luxurious fire woods I know of; it burns well, and in burning emits a delicious fragrance very generally admired' (Maiden, 1917). Latz (2004) reported that central Australian Aboriginal people used it for the same purpose, particularly for calming babies. However, from this reference it is unclear if an additive medicinal effect was intended, apart from having significant mythological significance. Due to the nature of other traditional use reports involving 'smudging' babies with ceremonial smoke for medicinal effects using other species (Sadgrove and Jones, 2013), it is possible that the same was done with *Callitris* species.

The second colonial use that resembles traditional Aboriginal use involved inhaling steamed needles for chills and pains (Low, 1990), which may be consistent with antiviral activity in flu cases, or general anaesthesia; but this is impossible to discern from these records alone. However, this medicinal application resembles the best known traditional Aboriginal use for this species (*Callitris endlicheri* and *Callitris glaucophylla*), which involved smouldering leaves over hot embers in a pit, with the patient positioned directly over the subsequent steamy smoke (Isaacs, 2000; Lassak and McCarthy, 2011; McKemey and White, 2011; Williams, 2011).

With regard to the traditional Aboriginal use of *Callitris* species in smoke fumigation treatment a number of targeted ailments have been described. Latz (2004) stated that *Callitris glaucophylla* was used medicinally by all central Australian tribes except the Pintupi and Pitjantjatjara. However, medicinal use modalities involving fumigation with smoke is not described by Latz (2004) but is mentioned by Williams (2011) as used for coughs and colds. Also in central Australia Barr (1988) adds that leafy branches were placed in a pit over hot fire embers, which was followed by profuse smoke fumigation. This was intended to produce a diaphoretic effect. The patient positions above the steamy smoke over the pit then shortly after lies down and goes to sleep. During sleep, subsequent sweating is allegedly consistent with improved health. Anecdotally, the traditional healers believed that the disease was removed in the sweat *per se* (Barr, 1988; Isaacs, 2000).

Antimicrobial activities of smoke extracts, demonstrated in this study, are consistent with traditional use of fumigation treatments for health complaints caused by microbial infection. Smoke extracts made using the first method (Fig. 1: Method A) using two fractions collected from the solvent and condenser, produced significant inhibitory activity against *Streptococcus pneumoniae*, which is associated with secondary lung infection. Greater activity was produced from the condenser fraction, with MIC values ranging from 400 to 700 μg/mL. The higher activity of this extract is interesting in the light of the similar temperature range required to produce condensation, in our study and on the human body or mucosal layer of the lungs. The occurrence of abietane dieterpenes in the smoke fraction may also be of relevance to this traditional medicinal application for treatment of colds and chest infections, in the light of demonstrated activity consistent with an anti-inflammatory response (Lin et al., 2010).

Traditional use modalities, using smoke from *Callitris* species, particularly *Callitris glaucophylla*, were also reported in other Australian states, such as New South Wales. In addition to these records, smoke fumigation treatments have also been accredited with restoring a sense of strength to the participant (McKemey and White, 2011). Non-medicinal traditional uses also involved ingesting the seeds as food (McKemey and White, 2011) and the leafy branches as a torch, which parallels Maiden's earlier comments (1917) about the easily combustible nature of *Callitris* timbers.

Topical uses of *Callitris endlicheri* and *C. glaucophylla* were also reported, but clarity regarding which species specifically treated which ailment, is lacking. In general, *Callitris glaucophylla* is the most frequently involved in topical use, medicinally employed by Aboriginal Australians in most areas, geographically corresponding to the species distribution. In a traditional setting, the needles and resin were either prepared as a poultice or extracted into water or animal fat, then applied for the treatment of various ailments (Latz, 2004), such as sores, scabies, rashes, body aches and pains, and also rubbed on the chest for colds (Low, 1990; Lassak and McCarthy, 2011; McKemey and White, 2011; Williams, 2011). Some references attribute the medicinal principle of topical treatment to antimicrobial activity, resulting from essential oil components such as α-pinene (Low, 1990) or geranyl acetate (Lassak and McCarthy, 2011). A contemporary modification of the topical medicinal aromatic preparation involves boiling the leaves to treat rashes or patted on the chest for colds (Barr, 1988; Isaacs, 2000). The *n*-butanol extract of this decoction did not produce significant antimicrobial activity in our current study.

On the other hand, methanol extracts produced significant inhibitory activity against Gram-positive bacterial species, most importantly *B. subtilis*, *S. aureus* and *S. pneumoniae*. The significance of this result, particularly against *S. aureus*, demonstrates the potential for a lipophilic extract (with respect to abietane diterpenes) or an aqueous extract (with respect to hydrophilic compounds visualised in bioautography) to treat Staph infections. Thus, in traditional Aboriginal Australian usage modalities, topical treatment using *Callitris glaucophylla* needles may have targeted these types of ailments.

Utilitarian uses of the resinous exudate are also recorded for these two species. Accordingly, the resin, also called gum, was used to secure stone tools to wooden implements (Clarke, 2007; McKemey and White, 2011), which may have involved initially mixing it with kangaroo dung to improve strength and durability (Latz, 2004).

5. Conclusion

Smoke extracts from needles of *Callitris* species contained a number of abietane diterpenes, some identified as ferruginol, pisiferal and pisiferol; some were not identified. These higher molecular mass compounds are not at all present in the essential oils but found in high concentration in solvent extracts. In terms of plant physiology, abietane dieterpenes may have been present in the resinous exudate, along with a host of other compounds. The importance of these abietane diterpenes in Australian Aboriginal medicinal use of *Callitris* species may be informed by Japanese medicinal use of *Chamaecyparis pisifera* (Cupressaceae).

It is not clear if these abietane dieterpenes were associated with the antimicrobial activities demonstrated in this study, but certainly other compounds, such as essential oils, cresol isomers, creosol and catechol, contributed to this effect. The unusually high antimicrobial activities produced by solvent extracts against Gram-positive species, particularly *B. subtilis* and *S. aureus*, was most likely from a hydrophilic compound, as demonstrated in bioautography. This unusual selectivity for these specific Gram-positive species was not demonstrated using smoke or essential oil condensates in antimicrobial assays. Thus, this hydrophilic compound is probably not volatile.

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The characterisation of medicinally significant compounds, along with demonstrated antimicrobial activities from smoke, solvent and essential oil condensates, conveys the truth about medicinal efficacies, which can be achieved using *Callitris* species; first described by Australian Aboriginal people. Further chemical and biological characterisation of the extracts examined in the current study may yield medicinally significant drugs. Firstly, novel abietane diterpenes can possibly be found in *Callitris endlicheri* needles. Secondly, hydrophilic compounds in *Callitris glaucophylla* needles may be further characterised and demonstrated to have significant antimicrobial properties. These works are currently being undertaken in our laboratory.

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References

Adams, R.P., 1999. Systematics of multi-seeded eastern hemisphere *Juniperus* based on leaf essential oils and RAPD DNA fingerprinting. Biochemical Systematics and Ecology 27, 709–725.

Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed. Allured Publishing Corporation, Illinois, United States, ISBN: 978-1-932633-21-4.

Areche, C., Theoduloz, C., Yanez, T., Souza-Brito, A.R., Barbastefano, V., de Paula, D., et al., 2008. Gastroprotective activity of ferruginol in mice and rats: effects on gastric secretion, endogenous prostaglandins and non-protein sulfhydryls. Journal of Pharmacy and Pharmacology 60 (2), 245–251.

Asuming, W.A., Beauchamp, P.S., Descalzo, J.T., Dev, B.C., Dev, V., Frost, S., et al., 2005. Essential oil composition of four Lomatium Raf. species and their chemotaxonomy. Biochemical Systematics and Ecology 33, 17–26.

Bailey, F.M., 1883. A Synopsis of the Queensland Flora, James C. Beal, Govt. Printer, Brisbane Australia, p. 497.

Baker, R.T., Smith, H.G., 1910. A Research on the Pines of Australia. Government Printer of NSW, Sydney.

Barr, A., 1988. Traditional Bush Medicines: An Aboriginal Pharmacopoeia. Greenhouse publications Pty Ltd., Richmond, Victoria, Australia.

Brecknell, D.J., Carman, R.M., 1979. Novel sesquiterpene lactones from *Callitris columellaris* heartwood. Australian Journal of Chemistry 32, 2455–2471.

Brophy, J.J., Goldsack, R.J., Forster, P.I., Copeland, L.M., O'Sullivan, W., Rosefelds, A.C., 2007. Chemistry of the Australian Gymnosperms. Part IX. The leaf oils of the Australian members of the genus *Callitris* (Cupressaceae). Journal of Essential Oil Research 19, 57–71.

Chassagne, D., Boulanger, R., Crouzet, J., 1999. Enzymatic hydrolysis of edible Passiflora fruit glycosides. Food Chem. 66, 281–288.

Clarke, P.A., 2007. Aboriginal People and Their Plants. Rosenberg Publishing Pty Ltd., Kenthurst Australia.

CLSI. 2009. Methods for dilution antimicrobial susceptibility testing for bacteria that grow aerobically; Approved Standard – Eight Edition. M07-A8 29(2). pp. 1–66.

Cribb, A.B., Cribb, J.W., 1981. Wild Medicine in Australia. William Collins, Pty, Ltd., Sydney.

da Silva, U.F., Borba, E.L., Semir, J., Marsaioli, A.J., 1999. A simple solid injection device for the analyses of *Bulbophyllum* (Orchidaceae) volatiles. Phytochemistry 50, 31–34.

Doimo, L., 2001. Azulenes, Costols and γ-lactones from cypress-pines (*Callitris columellaris*, *Callitris glaucophylla* and *Callitris intratropica*) distilled oils and methanol extracts. Journal of Essential Oil Research 13, 25–29.

Doimo, L., Fletcher, R., D'Arcy, B.R., 1999. Comparison of the γ-lactone content of oils and extracts from White Cypress Pine (*Callitris glaucophylla* Thompson and Johnson). Journal of Essential Oil Research 11, 415–422.

Espinoza, M., Santos, L.S., Theoduloz, C., Schmeda-Hirschmann, G., Rodriguez, J.A., 2008. New gastroprotective ferruginol derivatives with selective cytotoxicity against gastric cancer cells. Planta Medica 74, 802–808.

Guyot, C., Bouseta, A., Scheirman, V., Collin, S., 1998. Floral origin markers of chestnut and lime tree honeys. Journal of Agricultural and Food Chemistry 46, 625–633.

Harrison, B.M., Priest, F.G., 2009. Composition of peaks used in the preparation of malt for Scotch Whisky production – influence of geographical source and extraction depth. Journal of Agricultural and Food Chemistry 57 (6), 2385–2391.

Hasegawa, S., Kojima, T., Hirose, Y., 1985. Terpenoids from the seed of *Chamaecyparis pisifera*: the structures of six diterpenoids. Phytochemistry 24 (7), 1545–1551.

Hugo, W.B., 1978. Phenols: a review of their history and development as antimicrobial agents. Microbios 23 (92), 83–85.

Isaacs, J., 2000. Bushfood: Aboriginal Food and Herbal Medicine. Lansdowne Press, Sydney Australia.

IUPAC, 1997. Compendium of chemical terminology, 2nd ed. (the 'Gold Book'). Blackwell Scientific Publications, Oxford. XML on-line corrected version: ⟨http://goldbook.iupac.org⟩ (2006) created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. isbn:0-9678550-9-8. http://dx.doi.org/%2010.1351/goldbook.

Joulain, D., Koenig, W.A., 1989. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E.B. Verlag, Hamburg, Germany

Kocacalıskan, I., Talan, I., Terzi, I., 2006. Antimicrobial activity of catechol and pyrogallol as allelochemicals. Verlag der Zeitschrift für Naturforschung 61c, 639–642.

Kosyukova, L.V., Khorguani, T.V., 1989. Retention indices of diterpenes isolated from resins of coniferous trees. Journal of Analytical Chemistry of the USSR 44 (9, p2), 1309–1313.

Kuiters, A.T., Sarink, H.M., 1986. Leaching of phenolic compounds from leaf and needle litters of several deciduous and coniferous trees. Soil Biology and Biochemistry 18 (5), 475–480.

Kukic, J., Petrovic, S., Pavlovic, M., Couladis, M., Tzakou, O., Niketic, M., 2006. Composition of essential oil of *Stachys alpina* L. ssp *dinarica* Murb. Flavour and Fragrance Journal 21, 539–542.

Lai, W.-C., Song, C., 1995. Temperature-programmed retention indices for g.c. and g.c.–m.s. analysis of coal- and petroleum-derived liquid fuels. Fuel 74 (10), 1436–1451.

Lassak, E.V., McCarthy, T., 2011. Australian Medicinal Plants. Methuen Australia Pty Ltd., North Ryde.

Latz, P., 2004. Bushfires and Bushtucker: Aboriginal plant use in central Australia. IAD Press, Alice Springs, Australia.

Lee, K.-G., Lee, S.-E., Takeoka, G.R., Kim, J.-H., Park, B.-S., 2005. Antioxidant activity and characterisation of volatile constituents of beechwood creosote. Journal of the Science of Food and Agriculture 85, 1580–1586.

Lin, S., Zhang, Y., Liu, M., Yang, S., Gan, M., Zi, J., et al., 2010. Abietane and C20-norabietane diterpenes from the stem bark of Fraxinus sieboldiana and their biological activities. Journal of Natural Products 73 (11), 1914–1921.

Low, T., 1990. Bush Medicine: A Pharmacopoeia of Natural Remedies. Collins/Angus and Robertson Publishers, Australia.

Maiden, J.H., 1889. The useful native plants of Australia: Published by Compendium in 1975. Alexander Bros, VIC, Australia.

Maiden, J.H., 1917. Forestry Handbook. Part 2. Some of the Principal Commercial Trees of New South Wales: William Applegate Gullick. Government Printer, Sydney.

Mann, C.M., Markham, J.L., 1998. A new method for determining the minimum inhibitory concentration of essential oils. Journal of Applied Microbiology 84 (4), 538–544.

Mateo, J., Aguirrezabal, M., Dominguez, C., Zumalacarregui, J.M., 1997. Volatile compounds in Spanish paprika. Journal of Computational Analysis 10, 225–232.

McFarland, J., 1907. Nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. Journal of the American Medical Association 14, 1176–1178.

McKemey, M., White, H., 2011. Bush Tucker, Boomerangs and Bandages: Traditional Aboriginal Plant Use in the Border Rivers and Gwydir catchments: Border Rivers-Gwydir Catchment Management Authority. Greenridge Press, Toowoomba Australia.

Mueller, F.J.H.V., 1866. Fragment phytographiae Australiae 5 (40), 193.

NIST, 2011. NIST Chemistry WebBook – NIST Standard Reference Database Number 69. ⟨http://webbook.nist.gov/chemistry/⟩ (retrieved from: August 2012).

Olsen, R.A., Odham, G., Lindeberg, G., 1971. Aromatic substances in leaves of *Populus tremula* as inhibitors of mycorrhizal fungi. Plant Physiology 25, 122–129.

Oprava, A., Leach, D.N., Beattie, K., Connellan, P., Forster, P.I., Leach, G., et al., 2010. Chemical composition and biological activity of the essential oils from native Australian *Callitris* species. Planta Medica 76, SL_35.

Pino, J.A., Marbot, R., Vazques, C., 2001. Characterisation of volatiles in strawberry guava (*Psidium cattleianum* Sabine) fruit. Journal of Agricultural and Food Chemistry 49, 5883–5887.

Radulovic, N.S., Blagojevic, P.D., Palic, R.M., Zlatkovic, B.K., Stevanovic, B.M., 2009. Volatiles from vegetative organs of the paleoendemic resurrection plants *Ramonda servica* Panc. and *Ramonda nathaliae* Pan. at Petrov. Journal of the Serbian Chemical Society 74 (1), 35–44.

Re-Poppi, N., Santiago, M.R.-S., 2002. Identification of polycyclic aromatic hydrocarbons and methoxylated phenols in wood smoke emitted during production of charcoal. Chromatographia 55 (7/8), 475–481.

Sadgrove, N., Jones, G.L., 2013. A possible role of partially pyrolysed essential oils in Australian Aboriginal traditional ceremonial and medicinal smoking applications of *Eremophila longifolia* (R. Br.) F. Muell (*Scrophulariaceae*). Journal of Ethnopharmacology 147, 638–644.

Shaalan, E.A.-S., Canyon, D.V., Bowden, B., Younes, M.W.F., Abdel-Wahab, H., Mansour, A.-H., 2006. Efficacy of botanical extracts from *Callitris glaucophylla*, against *Aedes aegypti* and *Culex annulirostris* mosquitoes. Tropical Biomedicine 23 (2), 180–185.

Song, C., Lai, W.C., Madhusudan, R.K., Wei, B., 2003. Temperature-programmed retention indices for GC and GC–MS of hydrocarbon fuels and simulated distillation GC of heavy oils. In: Hsu, C.S. (Ed.), Analytical Advances for Hydrocarbon Research. Kluwer Academic/Plenum Publishers, New York, pp. 147–193 (Chapter 7).

Thompson, J., Johnson, L.A.S., 1986. *Callitris glaucophylla*, Australia's 'White Cypress Pine' – a new name for an old species. Telopea 2 (6), 731.

Waikedre, J., Vitturo, C.I., Molina, A., Theodoro, P.N.E.T., Silva, Md. R.R., Espindola, L.S., et al., 2012. Antifungal activity of the essential oils of *Callitris neocaladedonica* and *Callitris sulvata* heartwood (Cupressaceae). Chemistry and Biodiversity 9, 644–653.

Wanakhachornkrai, P., Lertsiri, S., 2003. Comparison of determination method for volatile compounds in Thai soy sauce. Analytical, Nutritional and Clinical Methods 83 (4), 619–629.

Watanabe, Y., Mihara, R., Mitsunaga, T., Yoshimura, T., 2005a. Termite repellent sesquiterpenoids from *Callitris glaucophylla* heartwood. Journal of Wood Science 51, 514–519.

Watanabe, Y., Mitsunaga, T., Yoshimura, T., 2005b. Investigating antitermitic compounds from Australian white cypress heartwood (*Callitris glaucophylla* Thompson et Johnson) against *Coptotermes formosanus* Shiraki. Journal of Essential Oil Research 17, 346–350.

Wei, Y., He, J., Qin, H., Wu, X.A., Yao, X., 2009. Determination of ferruginol in rat plasma via high-performacne liquid chromatography and its application in pharmacokinetics study. Biomedical Chromatography 23 (10), 1116–1120.

Wilkinson, J.M., Cavanagh, H.M.A., 2005. Antibacterial activity of essential oils from Australian native plants. Phytotherapy Research 19, 643–646.

Williams, C., 2011. Medicinal Plants in Australia. Gums, Resins, Tannin and Essential Oils, 2. Rosenberg Publishing Pty Ltd., Kenthurst Australia.

Xiao, D., Kuroyanagi, M., Itani, T., Matsuura, H., Udayama, M., Murakami, M., et al., 2001. Studies on constituents from *Chamaecyparis pisifera* and antibacterial activity of diterpenes. Chemical and Pharmaceutical Bulletin 49 (11), 1479–1481.

Xu, X., van Stee, L.L.P., Williams, J., Beens, J., Adahchour, M., Vreuls, R.J.J., et al., 2003. Comprehensive two-dimensional gas chromatography (GC × GC) measurements of volatile organic compounds in the atmosphere. Atmospheric Chemistry and Physics 3, 665–682.

Yatagai, M., 1997. 7. Miticidal activities of tree terpenes. Current Topics in Phytochemistry 1, 85–97.

Yatagai, M., Nakatani, N., 1994. Antimite, antifly, antioxidative, and antibacterial activities of pisiferic acid and its congeners. Journal of the Japan Wood Research Society 40 (12), 1355–1362.

Yatagai, M., Takahashi, T., 1980. New diterpenes from *Chamaecyparis pisifera*. Phytochemistry 19, 1149–1151.

Zhang, M.J., Li, D.S., Chen, B.J., 1992. Compositional studies of high-temperature coal tar by GC/FTIR analysis of light oil fractions. Chromatographia 33 (3–4), 138–146.

Zore, B.G., Thakre, Ad, Rathod, V., Mohan Karuppayil, S., 2011. Evaluation of anti-Candida potential of geranium oil constituents against clinical isolates of *Candida albicans* differentially sensitive to fluconazole: inhibition of growth, dimorphism and sensitization. Mycoses 54 (4), e99–e109.

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