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Chemical composition of essential oils of four *Eucalyptus* species and their phytotoxicity on silverleaf nightshade (*Solanum elaeagnifolium* Cav.) in Australia

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Abstract Phytotoxicity and chemical composition of essential oils from four selected *Eucalyptus* species in Australia were investigated. Essential oils had stronger inhibitory effects on germination and seedling growth of silverleaf nightshade (*Solanum elaeagnifolium* Cav.) when compared with a commercial eucalyptus oil and with 1,8-cineole. *E. salubris* oil had the highest inhibition index for silverleaf nightshade germination, root growth and shoot growth, while *E. spathulata* had the lowest inhibitory effect except root growth. Gas chromatography-mass spectrometry analysis revealed 56 compounds present in *E. salubris* oil, with 1,8-cineole (57.6 %), α-pinene (10.9 %) and *p*-cymene (8.3 %) predominant. *E. dundasii* oil contained 55 identified compounds with 1,8-cineole (65.5 %) and α-pinene (19.9 %) being the richest fractions. There were 56 compounds identified from *E. brockwayii* oil with α-pinene (31.1 %), isopentyl isovalerate (20.2 %) and 1,8-cineole (16.9 %) as the most abundant components. *E. spathulata* oil contained 60 compounds, predominantly 1,8-cineole (52.9 %) and α-pinene (31.0 %). Further study is required to determine the phytoxicity of the individual identified compounds on silverleaf nightshade and whether the observed phytotoxicity is attributable to a single compound or to the synergistic effects of several compounds.

Keywords *E. salubris* · *E. dundasii* · *E. brockwayii* · *E. spathulata* · Essential oil · Silverleaf nightshade · Herbicide · Allelopathy

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Introduction

Eucalyptus belongs to the myrtle (*Myrtaceae*) family. While typically native to Australia, a small number of species are indigenous to neighbouring countries, such as Papua New Guinea and Indonesia (Coppen 2002). Essential oils from eucalyptus have many traditional uses and potential commercial implications (Kandasamy et al. 2000; Zhang et al. 2010). They can be used as a folk medicine and have been reported to have a range of bioactivity, including antimicrobial, antiviral, fungicidal, insecticidal, anti-inflammatory, anti-nociceptive, anti-oxidant and phytotoxic activity (Duke 1983).

The phytotoxic activity of eucalyptus essential oils suggests that they may have potential commercial value as natural herbicides (Zhang et al. 2010). Setia et al. (2007) reported that volatile essential oils from *E. citriodora* were phytotoxic to the germination and growth of a number of weed species, such as *Bidens pilosa, Amaranthus viridis, Rumex nepalensis* and *Leucaena leucocephala*. Similarly, Ramezani et al. (2008) reported that essential oils from *E. nicholii* strongly inhibited the germination of *Amaranthus retroflexus, Portulaca oleracea* and *Acroptilon repens*. The herbicidal activity of eucalyptus essential oils against *Parthenium hysterophorus*, *Cassia occidentalis*, *Echinochloa crus-galli* and *A. viridis* has also been documented (Batish et al. 2004, 2006; Singh et al. 2006).

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) has become a serious problem in Australia, in particular in New South Wales, Victoria and South Australia (Stanton et al. 2009). It is a deep-rooted, summer-growing perennial weed of the Solanaceae family that is a declared noxious weed in several countries, including Australia, South Africa and approximately 20 states of the USA (OEPP/EPPO 2007; USDA-NRCS 2005). The management of this weed includes cultural, mechanical, chemical and biological controls (OEPP/EPPO 2007). However, the weed is very difficult to control, possibly due to the strong regenerative ability of the root system. In the absence of reliable and effective control options, alternative control options are needed for the effective management of this weed.

Field observations have identified that there is limited vegetation within the dripline of four *Eucalyptus* species: *E. salubris*, *E.dundasii*, *E. brockwayii and E. spathulata.* The presence of these *Eucalyptus* species in silverleaf nightshade infested roadside areas has assisted with the management of the weed. It is suspected that essential oils from the four special *Eucalyptus* species may play a role in suppressing silverleaf nightshade although the suppression may also be associated with other factors such as competition for resources. Therefore, this study was conducted to firstly determine the phytotoxicity of the above four eucalyptus essential oils against silverleaf nightshade, in comparison with a commercial eucalyptus essential oil, and 1,8-cineole, one of major components in most eucalyptus essential oils. Secondly, the chemical compositions of the essential oils from the four *Eucalyptus* species were determined and compared.

Methods and materials

Plant materials and chemicals

Approximately 2 kg of fresh leaves of *E. salubris*, *E.dundasi*, *E. brockwayii* and *E. spathulata* were collected from the field at Ungarie (Long. 146°55′41.33″, Lat. 33°35′53.06″), New South Wales (NSW), Australia. The leaves were then stored in a cool room (10 °C) before the extraction of essential oil. Seeds of silverleaf nightshade were collected in April 2008 from a silverleaf nightshade site at Culcairn (Long. 147°10′7.75″, Lat. 35° 35′38.11″), NSW. The seeds were dried and stored in a glass jar at the room temperature prior to the seed germination bioassays in November 2009. The seeds collected had 97 % viability in a tetrazolium assay. A commercial eucalyptus oil was purchased from a local super market (Woolworths, Australia) and 1,8-cineole was purchased from Sigma-Aldrich Pty. Ltd (Castle Hill, Australia).

Extraction of essential oils

Essential oils were extracted according to Batish et al. (2006) with some modifications. Three hundred grams of fresh leaves of eucalyptus leaves were cut into 5 mm strips and subjected to steam-distillation for 2.5 h using a Pyrex oil distillation apparatus with a flat bottom flask (2 L) containing 1,200 mL distilled water to generate steam. The volatile components from the leaves were condensed through a cooling tube. A separation funnel was used to collect the distilled essential oil, which was then dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C before use.

Bioassays of essential oils on weed germination and growth

A previous bioassay protocol (Batish et al. 2004) was adopted with slight modifications. Seeds of silverleaf nightshade were dipped in distilled water for 5 h prior to germination bioassays. Fifty seeds were placed in a 9-cm Petri dish lined with one layer of Whatman No.1 filter paper moistened with 5 mL of distilled water. To test the inhibitory effects of essential oils and the pure compound (1,8-cineole), an aliquot of 0, 10, 30, 90 and 270 μL essential oil were loaded using an Eppendorf micro pipette onto a piece of filter paper (2 × 2 cm) attached to the inner side of the cover of the Petri dish. The Petri dishes were then sealed with parafilm and maintained in a growth incubator with a diurnal temperature cycle of 25 °C in light and 15 °C in dark and a 12 h photoperiod. A randomized complete block design with three replicates was used. Seeds with >1 mm radical growth were considered as germinated and seedling length measured after 20 days of incubation.

Chemical analysis of essential oils

The essential oils were analysed by gas chromatography (GC)-mass spectrometry (MS) with the use of J & W DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) in a Varian 3800 gas chromatograph directly coupled to a Varian Saturn 2000 Ion Trap (ITD) mass spectrometer controlled by a Saturn GC/MS workstation (v5.2). Gas chromatography operating conditions followed those described by Adams (1995): 240 °C injector and transfer line temperature; 60–250 °C at 3 °C/min oven temperature, with a final hold time of 8.67 min at 250 °C (total run time 72.0 min); Helium carrier gas; 0.2 μL sample injection volume; 1:20 split ratio. Mass spectrometry acquisition parameters were: full scan with scan range 41–415 amu; 1.0 s scan time; 1 count threshold; AGC mode on; 5 microscans; 1.8 min filament delay. Column head pressure was adjusted to 13.0 psi.

Table 1 Inhibition potential of different essential oils and 1,8-cineole on the germination and seedling growth of silverleaf nightshade

Treatment	Germination Inhibition index (%)	Treatment	Root length Inhibition index (%)	Treatment	Shoot length Inhibition index (%)	Inhibition potential
E. salubris oil	73.0	*E. salubris* oil	82.0	*E. salubris* oil	75.7	Strong
E. dundasii oil	64.5	*E. dundasii* oil	77.7	*E. dundasii* oil	74.3	↓
E. brockwayii oil	58.9	*E. spathulata* oil	73.9	*E. brockwayii* oil	73.2	
E. spathulata oil	48.4	*E. brockwayii* oil	72.9	*E. spathulata* oil	71.2	
1,8-Cineole	41.9	1,8-Cineole	64.0	1,8-Cineole	58.4	
Commercial oil	38.4	Commercial oil	50.0	Commercial oil	49.0	Weak

Compounds were identified by comparing their Kovats indices (KI), retention times and mass spectra with Adams (1995), aided with NIST mass spectra library. Quantification of essential oil components (expressed as percentage of total peak area of chromatogram) was carried out by peak area normalisation measurements.

Statistical analysis

The dose–response data were subjected to the analysis of whole-range assessment proposed by An et al. (2005). The whole-range assessment considers overall effect/response across the whole range of application rates, instead of assessing the effect of each individual rate on test species. The program WESIA (Whole-range Evaluation of the Strength of Inhibition in Allelopathic-bioassay) developed by Liu et al. (2007) was used to calculate the inhibition index. The inhibition index is a summary of the overall biological response of an organism to a tested allelochemical or equivalent and provides a relative strength indicator of biological response. Large values indicate that the species is sensitive or that the allelochemical possesses strong allelopathic potential/biological activity, whilst small values indicate tolerance or weak potential/biological activity.

Principle Component Analysis (PCA) was used to analyze the chromatogram profiles and to provide supplementary analysis on the chemical differences between essential oils tested. The software for PCA followed Hammer et al. (2001).

Results

Bioassays of essential oils on weed germination and growth

All essential oils tested inhibited the germination of silverleaf nightshade, depending on the species (Table 1). The inhibition varied between the essential oils of different *Eucalyptus* species. The essential oil from *E. salubris* had the highest inhibitory activity on silverleaf nightshade germination, with a germination inhibition index of 73 %, whereas the commercial essential oil purchased from the market was the least, with an inhibition index of only 38 %. The essential oils of the four selected *Eucalyptus* species had a higher inhibition than that of the commercial essential oil or the pure 1,8-cineole. The inhibition potential was ranked in a decreasing order as *E. salubris* oil, *E. dundasii* oil, *E. brockwayi* oil, *E. spathulata* oil, 1,8-cineole and commercial oil based on the whole range assessment. These results are similar to the reported effects of other eucalyptus essential oils on weeds (Batish et al. 2004, 2006; Setia et al. 2007) although neither the phytotoxicity of eucalyptus essential oils on silverleaf nightshade nor the phytotoxic effects of essential oils from these four eucalyptus species on other weeds have been reported previously.

The inhibitory effect increased as the dose of the essential oil increased (Fig. 1). The germination of silverleaf nightshade was decreased by more than 50 % at a dose of 270 μL/dish for all oils.

The bioassays also showed that the seedling root growth of silverleaf nightshade was suppressed by all essential oils (Table 1, Fig. 2). Increasing essential oils dose levels resulted in higher inhibitory effects on silverleaf nightshade. *E. salubris* essential oil was the most inhibitory oil, reducing root growth by 84 % when applied at 10 μL/dish. The commercial essential oil showed the least inhibitory activity, causing only 41 % reduction in root length at the same dose and only 59 % reduction at 270 μL/dish. The inhibition potential was ranked in a decreasing order as *E. salubris* oil, *E. dundasii* oil, *E. spathulata* oil, *E. brockwayii* oil, 1,8-cineole and commercial essential oil (Table 1) based on the whole range assessment.

The essential oils also significantly suppressed the shoot growth of silverleaf nightshade seedling (Table 1, Fig. 3).

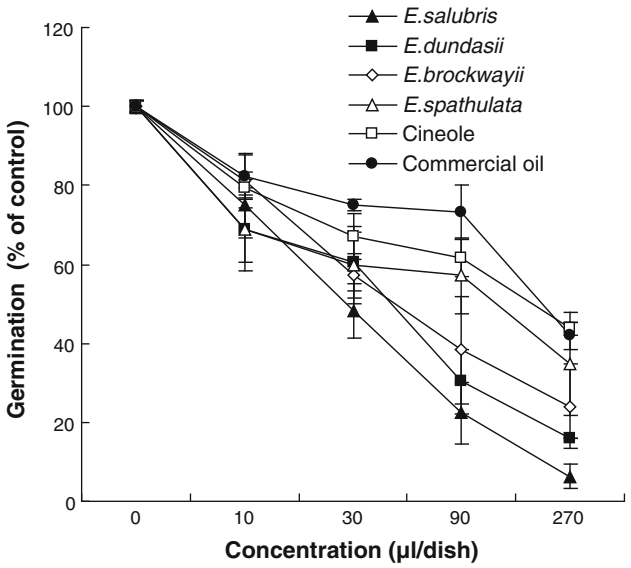

Fig. 1 Effect of essential oils from *E. salubris*, *E. dundasii*, *E. brockwayii* and *E. spathulata* as well as 1,8-cineole and commercial oil on germination of silverleaf nightshade

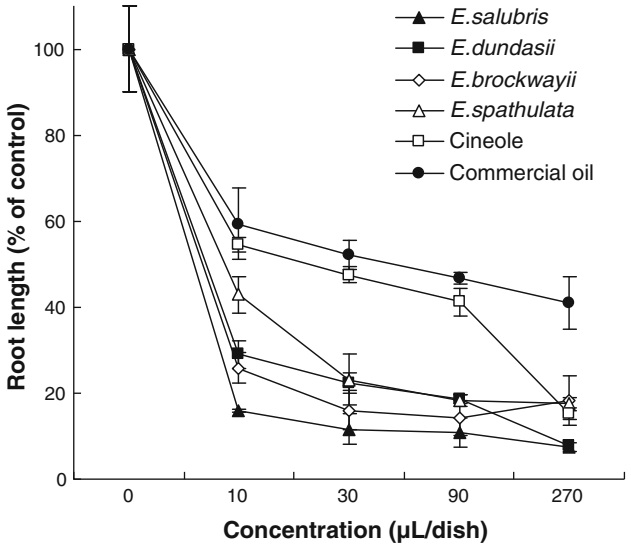

Fig. 2 Effect of essential oils from *E. salubris*, *E. dundasii*, *E. brockwayii* and *E. spathulata* as well as 1,8-cineole and commercial oil on root growth of silverleaf nightshade

This inhibition became more severe with increased dose used, but different degrees of inhibition were observed between the essential oils. The application of the essential oil of *E. salubris* at 10 µL/dish resulted in more than 80 % inhibition on the shoot growth of silverleaf nightshade, while the commercial essential oil had only 54 % reduction in the shoot growth even at the highest dose of 270 µL/dish. The inhibition potential was ranked in a decreasing order similar to the germination inhibition reported above.

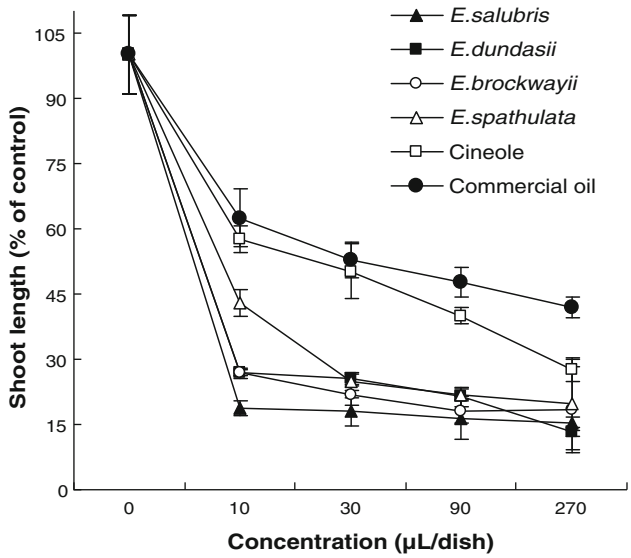

Fig. 3 Effect of essential oils from *E. salubris*, *E. dundasii*, *E. brockwayii* and *E. spathulata* as well as 1,8-cineole and commercial oil on shoot growth of silverleaf nightshade

Chemical analysis of essential oils by GC–MS

Each essential oil has a distinct chemical profile (Fig. 4). The composition of essential oils, the content of main compounds and ratio of each individual component varied considerably between the species (Table 2). This was further demonstrated by the comprehensive PCA analysis (Fig. 5), which showed that five essential oils were distinctly separated from each other by the PCA first principle component, accounting for 83 % of the total variance. 1,8-Cineole was the most abundant component for all essential oils except *E. brockwayii* oil. The selected *Eucalyptus* species in the decreasing order of 1,8-cineole content were *E. dundasii, E. salubris, E. spathulata and E. Brockwayii* (Table 2). The commercial eucalyptus oil contained a higher 1,8-cineole level than the four species tested.

A total of 55 compounds were identified in the essential oil extracted from the leaves of *E. dundasii*. The dominant components were 1,8-cineole (65.5 %), and α-pinene (19.9 %). This result did not quite agree with the previous work by Bignell et al. (1996a), who reported that 1,8-cineole content was 34.4 %. A number of factors could contribute to this variation such as the sub-species variant, sites and extraction method/time (Zhang et al. 2010). The essential oil of *E. salubris* consisted of 56 identifiable compounds, with the 1,8-cineole (57.6 %), α-pinene (10.9 %) and *p*-cymene (8.3 %) being the main component. The content of 1,8-cineole is a little higher than that in the previous report (Bignell et al. 1996b), in which the value is 48.8 %. There were 60 compounds identified in the essential oil of *E. spathulata*, with the predominant compounds being 1,8-cineole (52.9 %) and α-pinene (31.0 %).

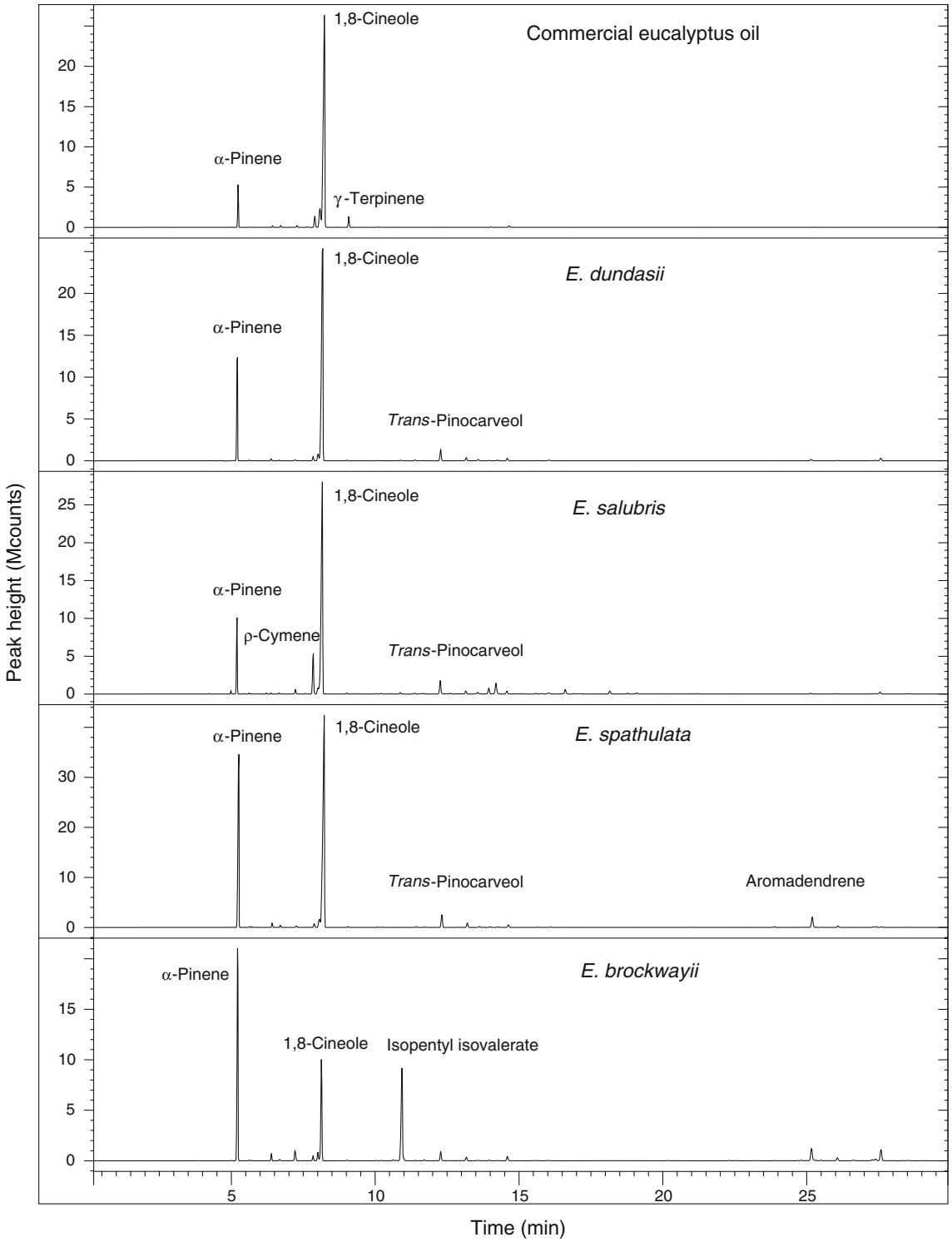

Fig. 4 GC-MS profile of essential oils from different *Eucalyptus* species

Our result was in agreement with the previous work (Fathi et al. 2009) but 37 more compounds were identified. In the essential oil of *E. brockwayii*, 56 compounds were identified with α-pinene (31.1 %), isopentyl isovalerate (20.2 %) and 1,8-cineole (16.9 %) as the most abundant components. Instead of 1,8-cineole, α-pinene was the dominant constituents. This composition pattern was similar to the work by Bignell et al. (1996b).

Table 2 Main compounds identified in essential oils and their relative percentage

Oils/Compound	1,8-Cineole	α-Pinene	Isopentyl isovalerate	*p*-Cymene	*trans*-Pinocarveol	Limonene	Pinocarvone	Compounds identified
1,8-Cineole	100	na	na	na	na	na	na	na
Commercial oil	77.0	7.8	–	3.1	–	7.0	–	28
E. dundasii	65.5	19.9	–	0.9	2.8	2	0.8	55
E. salubris	57.6	10.9	–	8.3	2.9	1.4	0.7	56
E. spathulata	52.9	31.0	–	0.7	2.4	2.1	0.9	60
E. brockwayii	16.9	31.1	20.2	0.8	1.8	1.5	0.8	56

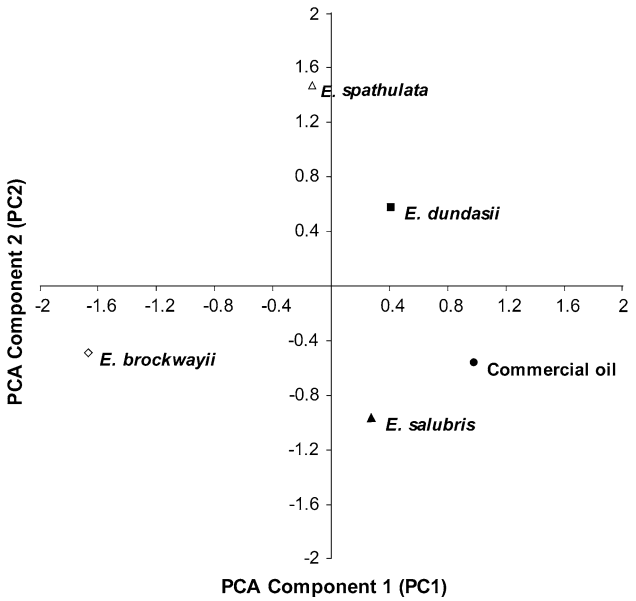

Fig. 5 PCA analysis of chemical profiles of the essential oils tested

Discussion

All essential oils tested inhibited the germination and seedling growth of silverleaf nightshade, but different degrees of inhibition were observed between the essential oils. The essential oils from *E. salubris* oil, *E. dundasii* oil, *E. brockwayi* oil *and E. spathulata* had stronger phytotoxic effect on silverleaf nightshade compared with the commercial eucalyptus oil. Among these four species, *E. salubris* oil had the highest inhibition index for silverleaf nightshade germination, root and shoot growth. Moreover, the bioactivity of the four essential oils on other weeds such as wild radish and barley grass was also under testing and more phytotoxic effects were found. This preliminary study supports essential oils playing a crucial role in suppressing understory growth of silverleaf nightshade within the driplines of the four selected *Eucalyptus* species in the field. The planting of suitable *Eucalyptus* species could be an alternative management strategy for the effective control of this intractable weed. *E. salubris* may serve as a potential source for developing a natural herbicide for the control of silverleaf nightshade and other weed species.

As one of major components found in the essential oils tested, 1,8-cineole could contribute to the bioactivity of oils tested as the herbicidal activity of 1,8-cineole has been demonstrated on other weeds (Singh et al. 2002; Romagni et al. 2000). 1,8-Cineole has been successfully used as a lead compound in the development of an morphogenetically active grass herbicide for use in broadleaf crops such as soybeans [*Glycine max* (L.) Merr.] (Baum et al. 1998). Our study also showed that 1,8-cineole inhibited the germination and seedling growth of silverleaf nightshade (Table 1, Fig. 1, 2, 3).

However, the inhibition index of 1,8-cineole on either germination or seedling growth of silverleaf nightshade was lower than that of the extracted essential oils (Table 1), and similar to the inhibition index of commercial oil which was composed of 77 % 1,8-cineole. The essential oil of *E. brockwayii* had the lowest content of 1,8-cineole (16.9 %), but higher activity than the commercial oil. Similarly, the best herbicidal activity was obtained with *E. salubris* oil, which had only a moderate 1,8-cineole content but the highest *p*-cymene content (8.3 %). These results suggest that the herbicidal activity of essential oils tested against silverleaf nightshade may not be associated solely with a single major compound, but may result from the synergistic effects of several bioactive compounds. Chemical and PCA analysis showed that the content of major compounds varied among oils tested, which may be responsible for the differences in phytotoxicity between these oils. The potential elevated phytotoxicity due to the synergistic effects of mixtures could assist in the development of natural herbicides in the future and would warrant further investigation.

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