Extraction Methods Effects on Composition and Toxicity of *Eucalyptus* Essential Oil

Mohammad Ebrahim Farashiani [1,2], Rita Muhammad Awang[1] Fatemeh Sefidkon [2], Dzolkhifli Omar [1], Mohammad Hassan Assareh [3] and Mawardi Rahmani [4],

[1.] Department of Plant Protection, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia.
rita@agri.upm.edu.my, EbrahimFarashiani@yahoo.com
[2.] Department of Plant Medicine, Research Institute of Forests & Rangelands, (RIFR), Tehran, Iran
[3.] Department of Biotechnology, Research Institute of Forests & Rangelands, (RIFR), Tehran, Iran
[4.] Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia

Abstract: The current investigation was designed to determine the influence of three extraction methods on the composition and insecticidal activity of essential oils extracted from *Eucalyptus globulus* and *E. camaldulensis*. The evaluated extraction methods were steam-distillation, steam water-distillation and hydro-distillation. Fumigant toxicity of the extracted essential oils was evaluated against *Sitophilus oryzae*. The GC and GC–MS methods were used for analyzing the oil chemical composition. Both *E. globulus* and *E. camaldulensis* showed significant differences in oil yield (w/w, based on dry weight) with direct steam distillation resulting in low oil yields (0.8%; 0.35%) compared to water distillation (2.35%; 2.22%) and water + steam distillation (2.03%; 1. 53%). We identified nineteen compounds in the essential oils of these species. 1, 8-Cineol (27.67-82%), α-Pinene (4.67-8.13%) and Limonene (2.49-10.53%) were the major components of the oils and the highest amount of 1, 8-Cineol (82%) was obtained with water distillation, while steam-distillation resulted in the lowest amount of Cineol (27%). The results of the toxicity study of the essential oils demonstrated that the highest toxicity (LC_{50} = 24.89 µL/L air) was observed against *S. oryzae* populations treated with oils extracted by water distillation. In conclusion, the extraction of *Eucalyptus* essential oils by hydro-distillation had some priorities over the oil extractions by the other methods because hydro-distillation extracted oil had the highest fumigant activity, high and fast-oil yields as well as high percentage composition of 1,8-Cineol.

[Farashiani ME, Muhammad Awang R, Sefidkon F, Omar D, Assareh MH, Rahmani M. **Extraction Methods Effects on Composition and Toxicity of *Eucalyptus* Essential Oil**. *Life Sci J* 2013;10(4):2401-2405]. (ISSN:1097-8135). http://www.lifesciencesite.com. 321

Keywords: Eucalyptus Essential oil; extraction methods; 1, 8-Cineol, fumigant toxicity, *Sitophilus oryzae*

1. Introduction

Eucalypt trees are evergreen and they belong to *Eucalyptus* genus, Myrtaceae family. *Eucalyptus* genus is native to Australia and there are more than 700 *Eucalyptus* species (Brooker and Kleinig, 2006). Eucalyptus trees are fast growing and they are planted in a wide range of climate for timber, pulp and wood-fuel crop.

They are also planted for their essential oils (Brooker and Kleinig, 2006). Most of the Eucalyptus trees (about 500 species of them) produce some types of essential oils. Based on the use of Eucalyptus essential oils, they are grouped into three types namely industrial, medicinal and perfumery (Boland et al., 1991). In the European Pharmacopeia, the Eucalyptus oil with more than 70% 1, 8-cineole has been used as medicinal products (Brooker and Kleinig, 2006; FAO, 1995). The essential oils of Eucalyptus are also important as they have insecticidal properties and have been used as natural pesticides (Haouel et al., 2010; Lee et al., 2004; Lee et al., 2000; Papachristos and Stamopoulos, 2004).

There are some factors that may influence the quality and quantity as well as the insecticidal properties of Eucalyptus essential oils and the method of oil distillation is probably one of the important factors. Water and steam-distillation, hydro-distillation (water-distillation) and steam-distillation are three main methods for extracting Eucalyptus essential oils (Boland et al., 1991; Handa et al., 2008). There is a considerable body of published studies describing the role of different distillation methods on the quantity, chemical composition and substance of essential oils in plants. For example Kiran et al. (2005) demonstrated that the amount of the rose-scented geranium essential oil (0.16–0.22%) obtained by hydro-distillation methods was more than steam-distillation extracted oil (0.06–0.18%) or oil extracted by water-steam distillation method (0.09–0.12%). Also, the used extraction method had significant influences on the oil component percentage (Kiran et al., 2005). The influence of extraction methods and stages of plant maturation on *Thymus kotschyanus* essential oil composition and content have also been reported. The most essential oil was extracted by hydro-distillation., while the low yield was extracted by steam-distillation. Furthermore, the highest oil yield was obtained by hydro-distillation method at the plant blooming stage (Sefidkon et al., 1999).

The research to date has tended to focus on the extraction method influences on the content and chemical composition of essential oils, rather than extraction method influences on the insecticidal activity of the extracts. There are no published research results highlighting the effect of the extraction method on the insecticidal activity of extracted essential oils. The objectives of this research were to evaluate the influence of distillation method (water-distillation, steam-distillation and water-steam-distillation) on the constitution, composition and insecticidal activity of essential oils from selected *Eucalyptus* spp. *In the best knowledge of the authors*, no research study has been found to investigate the effect of distillation method on chemical constitution and fumigant toxicity of these oils. Hence, in addition to identifying the chemical composition of selected Eucalyptus essential oil, the influences of the different extraction methods on insecticidal activity (fumigant toxicity) of the oils were investigated.

2. Material and Methods

Influence of extraction method on essential oil composition and content

Plant materials

We collected leaves of *E. globulus* and *E. camaldulensis,* from Mazanderan Province in the north of Iran. The collected plant materials were dried completely under laboratory conditions (23-24 °C and darkness). The dried materials were stored and were hydro-distilled to extract their essential oil.

Isolation procedure

The dried leaves (80 g) in three replicates were subjected to direct steam-distillation, water-and-steam distillation and water distillation for 3 h, based on method recommended by the European Pharmacopoeia (European Pharmacopoeia, 1983). Anhydrous sodium sulfate was used for the extracted oils dehydration. The oils reserved in sealed vials in refrigerator (2 °C).

Identification of chemical components

The extracted essential oils were analyzed by gas chromatograph (Shimadzu GC-9A) with a fused silica column with DB-5 coating and electron capture detector. Area normalization method was used for calculating the percentages of present compounds, without considering response factors. *GC-MS analysis* of the oils was also performed by Varian 3400 GC-MS system. This system was implemented with a DB-5 fused silica column. The components in the oils were identified by comparison of their mass spectra with those in a computer library or with authentic compounds and confirmed by comparison of their retention indices and mass spectra with data published in the literature (Adams, 1995; Davies, 1990; Shibamoto, 1987; Stenhagen et al., 1974). *n*-alkanes homologous series was used for calculating the compounds retention indices.

Influence of extraction method on essential oil fumigant toxicity

To determine the influence of extraction method on toxicity of essential oils, Toxicity of the extracted essential oils against *S. oryzae* were evaluated in the laboratory in consonance with methods described by Negahban et al. (2006 a) with some modifications.

Insect rearing

S*itophilus oryzae* were reared on whole rice and 3 days old adult insects were used in the fumigation bioassay test. The rearing containers were kept in laboratory conditions (with an 8:16 h light: dark cycle, 27 ± 1 °C and 65-±5% R.H).

Toxicity bioassay

The oil fumigant activity was determined using filter paper (2 cm) soaked with Eucalyptus oil with different doses. Concentrations of the oil tested on *S. oryzae* were 0, 25, 27.31, 30.41, 33.53, 37.28 and 40.78 µl per l air. The soaked filter paper was attached to the screw caps of 40 ml glass vial (Negahban et al., 2006a, b). The caps were screwed tightly on the vial containing ten adults (3-day-old) insects. Each dose treatment and control was repeated 4 times. Mortality was recorded 24 h after treatment. LC_{50} values were estimated by Probit analysis (Finney, 1971) using the SAS v. 9.1.3 Software package in Microsoft Windows 7. The toxicity of the 'insecticides' tested was compared based on a 95% confidence limit of LC_{50}. The LC_{50} values for each essential oil were considered to be significantly different from one another when 95% CL of the LC_{50} values failed to overlap (Robertson et al., 2007).

3. Results

Table 1 presents the Oil yields of *E. globulus* and *E. camaldulensis* obtained by different extraction methods.

Table 1. Oil yields of *E. globulus* and *E. camaldulensis* obtained by different methods of distillation

Distillation method	Means of oil yield (g)	Means of oil yield (g)
	E. globulus	*E. camaldulensis*
Hydro-distillation	2.35a ± 0.42	2.22a ± 0.39
Water- and steam-distillation	2.03a ± 0.34	1.53a ± 0.23
Steam-distillation	0.8b ± 0.15	0.35b ± 0.07

Means within columns followed by the same letters are not significantly different (P<0.01; Turkey's Comparison Test).

The color of the isolated oils was yellow. The analysis of variance demonstrated that the effect of extraction methods on content of *E. camaldulensis and E. globulus* essential oils was significant (Table 1). The minimum oil yield was extracted by steam-distillation and the maximum oil yield was obtained by hydro-distillation.

Furthermore, The main essential oil components of *E. camaldulensis* and *E. globulus* are illustrated in Table 2. Nineteen components were identified in the essential oils of *E. camaldulensis* and *E. globulus* extracted by the different distillation methods. The constituents are arranged in the order of their elution on the DB-5 column.

Table 2. Essential oil components in *E. globulus* and *E. camaldulensis* extract derived using different distillation methods

	Compound	RI*	W D* (%)	W-S D* (%)	S D* (%)
E. globulus	α-Pinene	950	5.43	4.67	6.20
	Limonene	1056	2.49	2.83	7.73
	1,8-Cineol	1066	78.23	82.08	51.39
	Bicyclogermacrene	1505	0.09	1.78	10.32
	Spathulenol	1550	4.28	0.06	0.52
	γ-Eudesmol	1640	-	0.45	1.47
	β-Eudesmol	1667	0.56	2.35	7.39
	α-Eudesmol	1680	2.79	0.44	1.78
E. camaldulensis	α-Pinene	950	6.33	7.49	8.13
	Limonene	1056	4.50	4.55	10.53
	1,8-Cineol	1066	68.75	71.18	27.67
	Bicyclogermacrene	1505	0.49	4.07	15.45
	γ-Eudesmol	1640	3.06	1.26	3.74
	β-Eudesmol	1667	0.59	6.10	16.48
	α-Eudesmol	1680	6.33	1.47	4.046

Note: RI, retention indices in elution order from DB-5 column; W D, Water Distillation; W-S D, Water-Steam Distillation; S D, Steam Distillation.

In both *Eucalyptus* species, the main essential oil constituents extracted by water- steam-distillation and water distillation were 1,8-Cineol (68-82%), α-Pinene (5.4-6%) and Limonene (2.49- 4.45%). The main essential oil components obtained by steam-distillation were 1,8-Cineol (27-51%), Bicyclogermacrene (10-15.4%), β-Eudesmol (7-16%), α-Pinene (5.4%) and Limonene (7-10%)

The results demonstrated that the effect of extraction methods on the quantity of the main components (Cineol, α-Pinene and Limonene) was significant. Water and water-steam-distillation resulted in higher amounts of 1, 8-Cineol (68-82%), while steam-distillation resulted in the lowest amount of Cineol (27%) but with largest amount of β-Eudesmol (7-16%).

Effect of distillation method on essential soil fumigant toxicity

Toxic effects of the essential oils extracted by different distillation methods are expressed as fifty percent lethal concentration (LC_{50}) for mortality of *S. oryzae* adults exposed to the essential oils. The results showed that *E. globulus* and *E. camaldulensis* essential oils were toxic to *S. oryzae* adults (Table 3).

Table 3. LC_{50} values of the extracted essential against *S. oryzae*

	Distillation methods	**LC_{50} (μL/L air) (Min-Max)**	**Groups**
E. camaldulensis	Water	26.92 (22.51- 24.89)	A
	Water-Steam	28.48 (22.27 - 25.95)	A
	Steam	36.25 (29.04 – 33.58)	B
E. globulus	Water	29.94 (24.18 – 27.43)	A
	Water-Steam	29.90 (22.59 – 26.80)	A
	Steam	34.05 (31.03 – 32.24)	B

Note: 95% lower and upper fiducial limits are shown in parenthesis; Means within columns followed by the same letters are not significantly different

Probit analysis showed that *S. oryzae* was highly sensitive to all examined essential oils. The maximum and minimum corresponding LC_{50} values were 24.89 and 36.25 μl/l airs, respectively (Table 3). There were considerable differences in toxicity effects against *S. oryzae* for the essential oils extracted by different distillation methods (Table 3). Highest toxicities were observed against *S. oryzae* populations when treated with oils extracted by hydro distillation and water + steam distillation methods. In the case of *E. camaldulensis*, the LC_{50} values of the essential oils extracted by hydro distillation method and steam + water distillation method were 24.89 and 25.95 μL/L air, respectively.

In the case of *E. globulus,* the essential oils extracted by hydro distillation method and steam + water distillation method were more toxic than the oils extracted by the steam distillation method, with LC_{50} values of the former two being 27.43 and 26.80 μL/L air, respectively (Table 3). In both *Eucalyptus* species, the oils extracted by steam distillation method were less toxic than the oils extracted by the other two methods. The LC_{50} values of steam distilled *E.*

camaldulensis and *E. globulus* essential oils were 36.25 and 32.74 µL/L air, respectively (Table 3).

4. Discussions

The amounts of oil yields were extracted by various distillation methods differed from each other. For example, the minimum oil yield was extracted by steam- distillation and the maximum oil yield was obtained by hydro-distillation (Table. 1). These results may be explained by the fact that compared with the other methods; the steam-distillation method has been more influenced by the conditions of the plant materials (for example type, mode of combination, mode of charging and grade of insulation. Similar results have been reported on the influence of extraction method on the constituents and content of essential oils of other plants (Kiran et al., 2005; Sefidkon et al., 2009). The differences in oil yields between the hydro distillation and water-steam distillation methods, were not significant and hence they are placed in the same group (Table 1).

The present work clearly demonstrates that the extraction method influences the quantity of 1, 8-cineole extracted (Table 2). The results showed that water and water + steam distillation methods extracted higher amounts of 1, 8-Cineol (68-82%), while steam-distillation resulted in the lowest amount of Cineol (27%) extracted (Table 2). In addition, the toxicity study also demonstrated that the different extraction methods had denoting effect in terms of LC50 values of the oils against *S. oryzae* (Table 3). Highest toxic effects were observed against *S. oryzae* populations treated with oils extracted by water distillation and water + steam distillation methods. Higher toxicity against the insect pest and higher amounts of 1, 8-Cineol were observed in oils extracted by water distillation and water + steam distillation. The 1, 8-Cineole is an important compound of *Eucalyptus* essential oils, and has been reported to play a key role in terms of insecticidal properties (Batish et al., 2008). Indeed, 1, 8 Cineole is a major component of *E. camaldulensis* and *E. globulus* essential oils and has been demonstrated as the leading cause of toxicity on several stored product insects. More recent literature has demonstrated that there is a relationship between 1, 8-Cineole content in *Eucalyptus* essential oils and their fumigant toxicity to stored product insects (Lee et al., 2004; Llusia and Penuelas, 2000; Negahban et al., 2006a; Obeng et al., 1997).

Additionally, the differences in toxicity of essential oils extracted by the different distillation methods are attributed to differences in the amount of 1, 8-Cineol present in the extracts**.** Hence, there was no significant difference in toxicity of essential oils extracted by water and steam + water distillation methods, as both extracts had about equal amounts of 1, 8-Cineol. On the other hand, the oils extracted by steam distillation method was less toxic than the oils extracted by the other two methods (water and steam + water distillation methods), and this was attributed to the lower amount of 1, 8-Cineol present in this extract. Similar results have been reported with The results obtained from the current study are in agreement with previous reports on influence of distillation methods on amount of 1,8-Cineol extracted in other aromatic plants (Batish et al., 2008; Kiran et al., 2005; Llusia and Penuelas, 2000; Sefidkon et al., 1999).

It is also noteworthy that the results presented in this current work are the first such published report on the influence of distillation method on fumigant toxicity of the eucalyptus essential oils extracted from *E. camaldulensis* and *E. globulus* against *Sitophilus oryzae*. the extraction of *Eucalyptus* essential oils by hydro-distillation had some priorities over the oil extractions by the other methods because hydro-distillation extracted oil had the high fumigant activity, high and fast-oil yields as well as high percentage composition of 1,8-Cineol.

It has been concluded that the current study enhances our knowledge about the effects of extraction method on chemical composition, content and fumigant toxicity of essential oils. The methods used for distillation of *Eucalyptus* essential oils may be applied to extract essential oils from other plants elsewhere in the world. It would be interesting to assess the influence of the different extraction methods on composition, content and insecticidal activity of extracts from other important aromatic plants.

Acknowledgements:

Foundation item: Authors are grateful to the Department of Plant protection, University Putra Malaysia and Research Institute of Forests & Rangelands, (RIFR) of Iran for financial support to carry out this work.

Corresponding Author:

Prof. Dr. Rita Muhammad Awang
Department of Plant Protection
Universiti Putra Malaysia
43400, UPM Serdang, Selangor, Malaysia.
E-mail: rita@agri.upm.edu.my

References

1. Adams RP. Identification of essential oil component by gas chromatography/mass spectroscopy. Allured Publishing Crop, Carol Stream, USA, 1995.

2. Batish DR, Singh HP, Kohli RK, Kaur S. Eucalyptus essential oil as a natural pesticide Forest Ecol. Manag. 256, 2166-2174, 2008.
3. Boland DJ, Brophy JJ, House APN. Eucalyptus Leaf Oils. Use, Chemistry, Distillation and Marketing. Inkata Press, Melbourne/Sydney, 1991.
4. Brooker, MIH, Kleinig, DA, Field Guide to Eucalyptus. vol.1. South-eastern Australia. Bloomings, Melbourne, 2006.
5. Davies, NW, Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. A. 503, 1-24, 1990.
6. European Pharmacopoeia. Vol. 1. Maissoneuve, SA, Sainte Ruffine, 1983.
7. FAO Eucalyptus oil. Chapter 5, Available online at Flavor and Fragrances of Plant Origin, Food and Agriculture Organization of the United Nations, Rome, Italy (1995) http://www.fao.org/docrep/v5330e/V5350e07.htm (accessed on 8.03.11),1995.
8. Finney DJ. Probit analysis. University Printing House, Cambridge, England, 1971.
9. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants.. United Nations Industrial Development Organization and the International Centre for Science and High Technology (ICS-UNIDO),, Trieste, Italy, 2008
10. Haouel S, Jemâa JMB, Khouja ML. Postharvest control of the date moth Ectomyelois ceratoniae using eucalyptus essential oil fumigation. Tunisian J. Plant Prot., 5, 201-212, 2010.
11. Kiran GDV, Babu V, Kaul K. Variation in essential oil composition of rose-scented geranium (Pelargonium sp.) distilled by different distillation techniques Flavor Frag J 20, 222-231, 2005.
12. Lee BH, Annis PC, Tumaalii F, Choi WS. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects J Stored Products Res 40, 553-564, 2004.
13. Lee SE, Choi WS, Lee HS, Park BS. Cross-resistance of a chlorpyrifos-methyl resistant strain of Oryzaephilus surinamensis (Coleoptera: Cucujidae) to fumigant toxicity of essential oil extracted from Eucalyptus globulus and its major monoterpene, 1,8-cineole. J Stored Products Res 36, 383-389, 2000.
14. Lusia J, Penuelas J. Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions Am J Bot 87, 133-140, 2000.
15. Negahban M, Moharramipour S, Sefidkon F. Chemical Composition and Insecticidal Activity of *Artemisia scoparia* Essential Oil against Three Coleopteran Stored-Product Insects J Asia Pacific Entomol 9, 381-388, 2006a
16. Negahban M, Moharramipour S, Sefidkon F. Insecticidal Activity and Chemical Composition of *Artemisia sieben* Besser Essential Oil from Karaj, Iran J Asia Pacific Entomol 9, 61-66, 2006b.
17. Obeng OD, Reichmuth CH, Bekele J, Hassanali A. Biological activity of 1, 8 cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored product beetles J Appl Entomol 121, 237-243, 1997
18. Papachristos DP, Stamopoulos DC. Fumigant toxicity of three essential oils on the eggs of Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae) J Stored Products Res 40, 517-525, 2004
19. Robertson JL, Russel RM, Perisler HK, Savin, NE. Bioassay with Arthropods, 2 ed CRC Press, London, 2007.
20. Sefidkon F, Bahmanzadegan A, Assareh MH, Abravesh Z. Seasonal variation in volatile oil of eucalyptus species in Iran J *Herbs Spices* Med *Plants* 15, 106-120, 2009.
21. Sefidkon FM, Dabiri M, Bidgoly AR. The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Thymus kotschyanus* Boiss & Hohen, Flavour Frag J 14 405-408, 1999.
22. Shibamoto T. Retention indices in essential oil analysis In P Sandra & C Bicchi (Eds), Capillary gas chromatography in essential oil analysis (pp 259–274) Walter Huething Verlag, New York, NY, 1987
23. Stenhagen E, Abrahamson S, MacLafferty FW. Registry of Mass Spectral Data J Wiley & Sons, New York, 1974.

11/12/2013