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Essential Oil Chemical Constituent Analysis of *Cinnamomum iners*

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Abstract: The leaves and stems of *Cinnamomum iners* Reinw. ex Blume var. Subcuneatum (Miq) W.K. Soh (*C. iners*) were collected from Forest Research Institute Malaysia (FRIM) as the sample for this study. A large-scale steam distillation method was used to extract oil from 47 kg of the sample; yielding 0.063% essential oil. The *C. iners* essential oil was qualitatively analysed using Gas Chromatography-Mass Spectrometry (GC-MS) and reconfirmed using the Kovats Index (KI). The analysis showed that the main components of *C. iners* oil consists of geraniol (63.65%), linalool (19.42%), (E)-caryophyllene (4.80%), geranyl propanoate (3.51%), (E)-phytol acetate (1.91%), dill apiole (1.36%), β-selinene (1.01%), α-selinene (0.98%), β-pinene (0.98%), (E)-nerolidol (0.64%), α-pinene (0.63%), (E)-β-ocimene (0.60%) and α-humulene (0.52%). A quantitative analysis of linalool (from the extracted essential oil) was conducted using a Gas Chromatography-Flame Ionisation Detector (GC-FID) according to the Internal Standard Method (ISM). The linalool concentration was found to be 327 351 ppm.

Key words: *C. iners*, essential oils, Kovats index, Gas Chromatography-Mass Spectrometry (GC-MS), gas Chromatography-Flame Ionisation Detector (GC-FID)

INTRODUCTION

Cinnamomum iners (*C. iners*) is grown in the wild in Malaysia, India, Myanmar, Indonesia, Thailand, Singapore, Brunei and the Philippines, especially in the lowlands (Mustaffa *et al*., 2013). This species has several names based on where it is found, for example, it is known as Opchoei in Thailand (Jacobsen and Salguero, 2014), Sinkozi in Myanmar (Watt, 2014) and Gerpa or Nggepak (Quattrocchi, 2016) in Indonesia. The Malays call this species Medang while in the wastern world, it is more commonly known as Wild Cinnamon (Ng *et al*., 2011).

C. iners comes from the lauraceae family. Its medium-sized evergreen tree grows up to 24 m (Wiart, 2006) and has a bushy and rounded crown. New growth start with pink leaves that gradually fade to cream, yellowish green and then dark green. The thin leaves have three longitudinal veins and are leathery to the touch as they contain petiole with short hairs (Wiart, 2006). The leaves and stems emit a cinnamon smell when crushed. The light cream yellow *C. iners* flower is bisexual and arranged in auxiliary groups or terminal panicles. The flower produces a rancid and waxy smell to attract small beetles and hoverflies that act as pollinators. The fruit of this plant is round or ellipsoid and is berry-shaped. The colour of the fruit is initially green and then turns blue-black when it matures.

The nice smell of the *C. iners* oil indicates that it contains volatile compounds that produce pleasant smells. Previous research has shown that oil from the *C. iners* leaves originating from Thailand contains linalool, cadinol, α-cadinol, viridiflorol, caryophyllene, caryophllene oxide, eugenol, α-pinene and β-pinene. The major component in the oil of the leaves is 30-50% linalool (Phutdhawong *et al*., 2007). Linalool or 3, 7-dimethylocta-1, 6-dien-3-ol at a 99% composition has a pleasant scent. This is due to its terpene alcohol content

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that is naturally found in many flowers, fruits and spices. Other than *C. iners*, there are about 200 plant species that contain linalool, for instance Elettaria cardamomum from the Zingiberaceae famil (Ozek *et al*., 2010), Citrus bergamia from the Rutaceae famil (Kuwahata *et al*., 2013), Lippia alba from the Verbenaceae family (Costa *et al*., 2014) and Coriandrum sativum from the Apiaceae family (Duarte *et al*., 2016). A previous study proved that linalool has anti-inflammatory and antinociceptive propertie (Kuwahata *et al*., 2013). In addition, linalool-rich essential oils from Ocimum basilicum and Coriandrum sativum also have antimicrobial properties (Duman *et al*., 2010).

In this study, a large-scale steam distillation method was used to extract the Essential Oils (EOs) from the *C. inres* plant. The distillation method prevents the loss of volatile substances during the extraction process. This is due to its closed system that allows for the extraction of Eos (Kamaruddin *et al*., 2016) in the best possible way. This study used a qualitative approach to analyse the samples, i.e., via Gas Chromatography-Mass Spectrometry (GC-MS). The Kovats Index (KI) was also used to confirm the validity of the results generated from the GC-MS analysis. A Gas Chromatography-Flame Ionisation Detector (GC-FID) analysis and an Internal Standard Method (ISM) approach were employed for the determination of linalool concentration in the oil sample. Fenchone was chosen as the internal standard because in the chromatogram, the standard peak would appear with a baseline resolution before the peak of interest. In addition there will be no overlap between fenchone and the other compound peaks in the EO sample.

MATERIALS AND METHODS

Plant material: The leaves and stems of the *Cinnamomum iners* Reinw. ex Blume var. Subcuneatum (Miq) W.K. Soh (*C. iners*) were collected in a large-scale amount of about 47 kg from Forest Research Institute Malaysia (FRIM), Kepong, Selangor and used as the specimen for this study.

Extraction and isolation of oils: Oil extraction was conducted using a large-scale steam distillation method in a 100 kg container. Water was poured at the bottom of the container until it reached a marked level after which a lid with holes was used to cover it. The collected leaves and stems were then inserted into the closed container. Throughout the process, the container was ensured to have no leakage to prevent the loss of essential oils. The container had already been connected to a pre-setup condenser. Next, the sample was kept in a heating process for 8 h. From the distillation, a cloudy liquid was obtained, composed of a mixture of water and essential oils. The essential oil of the *C. iners* is miscible with water due to its density properties. The distillate was then extracted using hexane. Anhydrous sodium sulphate (Na_2SO_4) was used to dry the extracted essential oil. Finally, the oil was kept at 4°C for the GC-MS test and GC-FID analysis.

Gas Chromatography Flame Ionisation Detector (GC-FID) analysis: The *C. iners* oil was quantified using a GC-2010 Plus Shimadzu with a HP-5MS capillary column (30 m×0.25 mm, film thickness of 0.25 μm). The temperature for the injector and the detector was set at 250°C. The column temperature was programmed at 60°C for 10 min and then ramped up at a rate of 3°C/min up to 230°C. The flow rate of the carrier gas (helium), hydrogen and compressed air was set at 10, 40 and 400 mL/min, respectively. Finally, a sample of 1.0 μL was injected using a split mode (split ratio of 1:50).

Gas Chromatography Mass Spectrometry (GC-MS) analysis: The *C. iners* oil was analysed using an agilent-technologies 7890A/5975C MSD with a HP-5MS capillary column (30 m×0.25 mm, film thickness of 0.25 μm). The temperature for the injector and detector was set at 250°C. The column temperature was programmed at 60°C and increased at a rate of 3°C/min up to 230°C. The flow rate of the carrier gas (helium) was 10 mL/min. Then, 1.0 μL of the sample was injected using a split mode (split ratio of 1:50). The constituents of the oil were further identified and verified using mass spectra.

Compound identification: From the mass spectra obtained, the constituents were further determined using the Kovats Index (Hubschmann, 2015). Based on the peaks and retention times from the chromatogram, the retention index was calculated using the given formula. The oil constituents were identified by comparing their retention indices with values from the literature. A further comparison of their mass spectral data with those from Wiley's mass spectral data base is elaborated in the following section.

Quantification of linalool: A set of 1.40 mL standard solutions was prepared with linalool concentrations of 9 000, 27 000, 45 000, 63 000 and 81 000 ppm spiked with 100 μL of 97% fenchone. Then, the mixture was analysed using the GC-FID method. A calibration graph for the peak area ratio of linalool over fenchone was plotted against the standard concentration ratio.

The 200 μL of the *C. iners* oil sample was pipetted into a 1.5 mL glass vial and 100 μL of the fenchone was spiked into the sample and diluted with n-hexane to 1.40 mL of the volume. The sample mixture was then subjected to a GC-FID analysis. The concentration of linalool was then determined directly from the calibration curve.

RESULTS AND DISCUSSION

Percentage yield of EO: The large-scale steam distillation of 47 kg of fresh leaves and stems of the *C. iners* yielded 0.063% EO. Fresh leaves were used in this extraction process instead of dried leaves because the total essential oil would have decreased during the drying process (Figiel *et al*., 2010). EO yield percentage was calculated using a dry weight basis. The volume of oil extracted from the distillation was 9.8 mL. The average moisture content obtained from 3.00 g of leaves and stems was 2.0 mL. The moisture content was determined using the toluene distillation method (Pimentel *et al*., 2006).

Steam distillation was used in this study because of its high yield production. For instance, EO yield using steam distillation is higher than that of the hydro distillation method (Charles and Simon, 1990). The operator would also be in full control of this method. It is also a rapid process that prevents thermal degradation and produces oil of acceptable quality.

Identification of the constituents of *C. iners* essential oil: The *C. iners* oil constituents were determined using GC-MS supported by a KI analysis. A series of retention times of saturated hydrocarbons were used to calculate the KI. Table 1 shows the retention time of the saturated hydrocarbon peaks that appear in the chromatogram.

The most abundant compound in the oil of *C. iners* leaves and stems was geraniol ($C_{10}H_{18}O$) with a percentage composition of 63.65% followed by linalool ($C_{10}H_{18}O$) at 19.42% composition, representing the second largest peak in the chromatogram. Both compounds dominated the composition percentage in the EO.

These compounds are also structural isomers. Other compounds that can be found in the EO of *C. iners* were α-pinene, β-pinene, (E)-α-ocimene, geranyl propanoate, (E)-caryophyllene, α-humulene, β-selinene, α-selinene, (E)-nerolidol, dill apiole and (E)-phytol acetate. Table 2 shows the composition of all identified compounds in the *C. iners* oils obtained via the GC-MS and GC-FID methods.

Quantification of linalool: Linalool was chosen for quantification because it was one of the most abundant compounds in the *C. iners* EO. Fenchone proved to be a perfect internal standard in this determination because it does not overlap with linalool or any other existing compounds in the oil sample. Moreover, fenchone was also absent in the sample. The result of the analysis is presented in Table 3. A calibration curve of the peak area ratio of linalool over fenchone versus concentration of linalool is displayed in Fig. 1. The concentration of linalool was found to be 327 351 ppm.

Table 1: Standard retention time of hydrocarbon

No. of carbon	Retention time (min)
C_8	3.585
C_9	6.260
C_{10}	12.233
C_{11}	18.929
C_{12}	24.758
C_{13}	30.078
C_{14}	34.964
C_{15}	39.235
C_{16}	43.370
C_{17}	47.336
C_{18}	50.791
C_{19}	54.386
C_{20}	57.709
C_{21}	61.070
C_{22}	64.202
C_{23}	67.118

Table 2: Identified chemical compounds and compositions (%) in the *C. iners* oil based on the GC-MS, KI and GC-FID analysis

Compound	RI	Composition (%)	Identification
α-Pinene	0922	0.63	MS,KI
β-Pinene	0964	0.98	MS,KI
(E)-β-Ocimene	1043	0.59	MS,KI
Linalool	1103	19.42	MS,KI
Geraniol	1269	63.65	MS,KI
Geranyl propanoate	1379	3.51	MS,KI
(E)-Caryophyllene	1409	4.80	MS,KI
α-Humulene	1440	0.52	MS,KI
β-Selinene	1475	1.01	MS,KI
α-Selinene	1484	0.98	MS,KI
(E)-Nerolidol	1555	0.64	MS,KI
Dill apiole	1619	1.36	MS,KI
(E)-Phytol acetate	2105	1.91	MS,KI

Table 3: Data from the chromatogram of the internal standard method analysis

Concentration ratio	Peak area ratio
0.22	0.08
0.65	0.21
1.09	0.38
1.52	0.46
1.96	0.60

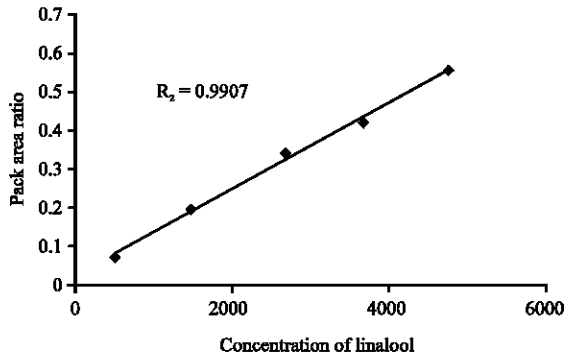

Fig. 1: Graph of the peak area ratio against concentration of linalool

CONCLUSION

A large-scale steam distillation method was successfully employed in the extraction of essential oil

from the investigated sample of *C. iners* in this study. It was found that 47 kg of leaves and stems of the *C. iners* plant was able to produce 0.063% of essential oil. Seven major volatile compounds were identified as making up >1% of the oil composition. These compounds are geraniol (63.65%), linalool (19.42%), (E)-caryophyllene (4.80%), geranyl propanoate (3.51%), (E)-phytol acetate (1.91%), dill apiole (1.36%) and β-selinene (1.01%). The exact concentration of linalool in the *C. iners* essential oil was determined to be 327 351 ppm.

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