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Major chemotypes and antioxidative activity of the leaf essential oils of *Cinnamomum osmophloeum* Kaneh. from a clonal orchard

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Abstract

Essential oils of 92 cutting clones from a clonal orchard of *Cinnamomum osmophloeum* Kaneh. were obtained by hydrodistillation and characterised by gas chromatography–mass spectrometry. Our results showed that the yields of essential oils ranged between 0.09% and 2.65% (vol/fresh wt). The constituents of essential oils varied among samples. The major chemotypes classified in the individual cutting clones were cinnamaldehyde (50 plants, representing 50–95% of the total volatiles), linalool (1 plant, 73.3%), β-cubebene (2 plants, 59.4% and 78.7%), and cinnamyl acetate (1 plant, 61.8%). The antioxidant activities of the four chemotypes were determined using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The antioxidant activities of the essential oil decreased in the order of cinnamyl acetate > cinnamaldehyde > β-cubebene > linalool. Indigenous cinnamon oil extract showed a good free radical-scavenging capacity at all concentrations studied, except at 2 μg/ml. The scavenging activity increased with increasing concentration of the extract. The capability of the four essential oil chemotypes to reduce the stable radical, DPPH, to DPPH-H was assayed by a decrease in the IC_{50} values of 10.4 (cinnamyl acetate type) to 29.7 (linalool type) μg/ml. These results suggest that the leaf essential oil of *C. osmophloeum* possesses chemical compounds with antioxidant activity which can be used as natural preservatives in food and/or by the pharmaceutical industry. Trees in this plantation which can be used for further propagation for the production of chemotypes of interest were identified.
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1. Introduction

Cinnamomum belongs to the family Lauraceae, and is a large genus, many species of which yield a volatile oil on distillation. The composition of the oil, and therefore, its value and the use to which it can be put, depends very much on the species that are distilled as well as the parts of the plant that are utilized (Chericoni, Prieto, Iacopini, Ciono, & Morelli, 2005). Most of the chemical components of essential oils are terpenoid compounds, including monoterpenes, sesquiterpenes, and their oxygenated derivatives. These low-molecular weight compounds easily diffuse across cell membranes to induce biological reactions (Chao et al., 2005). Previous studies demonstrated the bioactivity of these forest products, such as their antimicrobial, antifungal, antitermitic, and mosquito larvicidal properties, as well as their use in traditional folk medicine (Chang, Chen, & Chang, 2001; Sahin et al., 2004; Singh & Marimuthu, 2006; Tuberoso et al., 2005; Wang, Chen, & Chang, 2005). Furthermore, leaf essential oils of both cinnamaldehyde and cinnmaldehyde–cinnamyl acetate types

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of *Cinnamomum osmophloeum* have excellent inhibitory effects against white-rot fungi, *Rhizoctonia solani*, and other antifungal properties have been reported (Cheng, Liu, Hsui, & Chang, 2006; Lee, Cheng, & Chang, 2005).

Essential oils and various extracts of plants are commonly used in the food processing industry because of their special aromas and secondary metabolites. Exploration of the potential use of the leaf essential oils from *C. osmophloeum* has been advocated, since the chemical constituents of oils from *C. osmophloeum* are similar to the well-known bark oils from *C. cassia* (Chang et al., 2001; Cheng et al., 2006; Lee et al., 2005; Wang et al., 2005). Antioxidants have been widely used as food additives to provide protection against the oxidative degradation of foods by free radicals, or to prolong the storage stability of foods (Mathew & Abraham, 2006). Free radicals and other reactive oxygen species (ROS) are continuously generated via normal physiological processes, more so in stressful conditions. In plant cells, oxidative stress reactions are associated with toxic free radicals from the reduction of molecular oxygen to superoxide radicals (O_2^-), singlet oxygen ($^{\cdot}O_2$), hydroxyl radicals ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), and peroxyl radicals ($ROO^{\cdot}$) (Chang, Kim, Hwang, Choi, & Kim, 2002). Antioxidant activity plays a major role in maintaining the balance between the production and elimination of free radicals. The harmful action of free radicals can, however, be blocked by antioxidant substances which scavenge and detoxify free radicals in an organism. Current research into free radicals has confirmed that foods rich in antioxidants play essential roles in the prevention of cardiovascular diseases, cancers, and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases, as well as cellular and cutaneous aging (Leong & Shui, 2002; Tepe, Daferera, Sokmen, Polissiou, & Sokmen, 2004; Tepe, Sokmen, Sokmen, Daferera, & Polissiou, 2005). Indigenous cinnamon essential oil and its constituents have been studied in different systems for raw and processed food preservation, but few studies have dealt with the content, ability, capacity, and function of the antioxidant components.

In 1985, the former head of the Forestry Department, Dr. Ta-Wei Hu, at Chinese Culture University, established an indigenous cinnamon clonal orchard, at the Hwa-Lin University Experimental Forest in Taipei County, with cuttings of trees from 13 natural populations from central, eastern and southern regions of Taiwan. These cuttings were selected based on their taste in the mouth which indicated high levels of cinnamaldehyde. However, the actual amounts of cinnamaldehyde produced by those plants have not been evaluated by laboratory procedures, and therefore, their true value for cinnamaldehyde production is unknown. In this context, the objectives of this study were to (1) investigate the chemical composition of the leaf essential oil of *C. osmophloeum* plants from this clonal orchard; and (2) determine the antioxidant 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities of the essential oil of different chemotypes of *C. osmophloeum*.

2. Materials and methods

2.1. Plant material

Indigenous cinnamon (*C. osmophloeum* Kaneh.) is a tree that grows in Taiwan's natural hardwood forests at elevations of 400–1500 m (Chang & Cheng, 2002; Fang, Chen, & Cheng, 1989). The leaves of 92 individual *C. osmophloeum* trees (cutting clones) were harvested in July 2003 from a *Cinnamomum* clonal orchard at the Chinese Culture University's Hwa-Lin Experimental Forest in Taipei County. Unfortunately, the original records of where these indigenous cinnamon cuttings specifically came from among 13 populations distributed in central, southern and eastern regions of Taiwan were lost. The cut leaves were brought to the laboratory and washed, then air-dried of water on the surface of leaves.

2.2. Essential oil extraction and isolation

Twenty grams of fresh sample was homogenised and hydrodistilled with a Clevenger-type apparatus for 2 h. The essential oils were dried over anhydrous sodium sulfate and, after filtration, stored at −10 °C until treated and analyzed (Chang & Cheng, 2002). The essential oil content was determined volumetrically on a fresh weight basis. These experiments were run in triplicate.

2.3. Gas chromatography (GC)

The essential oil was analyzed using a Hewlett–Packard 5890SeriesII gas chromatograph (GC) equipped with a flame ionization detector and an HP-20M column (50 m × 0.32 mm, with a 0.3 μm film thickness). Injector and detector temperatures were set to 220 and 250 °C, respectively. The injection volume was 1 μl with a split ratio of 10:1. Helium was used as carrier gas at a flow rate of 1 ml/min. The oven temperature was held at 50 °C for 2 min, then programmed to rise to 200 °C at a rate of 2 °C/min.

2.4. GC/mass spectrometry (MS)

The essential oil constituents were quantitated under the same conditions with GC using an HP 5890SeriesII GC equipped with an HP 5973A mass selective detector in electron impact mode (at 70 eV). The injector and mass spectrometry (MS) transfer line temperatures were set to 250 and 265 °C, respectively. The identification of individual compounds was based on comparisons of their relative retention times with those of authentic samples on a CP-Wax 52BC capillary column (60 m × 0.25 mm, with a 0.25 μm film thickness) and by matching of their mass spectra with those obtained from internal standards. All standards were purchased from Sigma–Aldrich (St. Louis, MO). Essential oil components were also identified by computer matching against the Wiley GC–MS library. The relative percentages of the oil constituents were

expressed by peak area normalisation. *n*-Alkanes were used for the calculation of the Kovats indices. The experiment was performed twice independently to compare the reproducibility of the GC/MS analysis. Details of the GC and GC/MS procedures are described by Adams (2001).

2.5. DPPH free radical-scavenging assay

Involvement of the antioxidant system in regulation of free-radical metabolism was examined by measuring variations in the antioxidant ability. In this study, the free radical-scavenging ability of the *C. osmophloeum* was measured using the protocols described by Shimada, Fujikawa, Yahara, and Nakamura (1992) Yoshiki, Kahhara, Sakabe, and Yamasaki, 2001. Briefly, essential oil was dissolved in 1 ml of 100 mM acetate buffer (pH 5.5) and 2 ml of methanol. Final concentrations of essential oils were 2, 4, 8, 12 and 20 μg/ml. An aliquot of 0.2 ml of the methanolic extract was added to 0.5 ml of a 10 mM DPPH solution freshly prepared in methanol. The mixture was left in the dark for 30 min at room temperature, and decolorization of DPPH-donated H^+ was followed by measurement of the absorbance at 517 nm. DPPH radical-scavenging activity was calculated from the absorption according to the following equation:

$$\text{DPPH radical-scavenging activity } (\%) = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The extract concentration providing 50% inhibition (IC_{50}) was calculated from a graph plotting the inhibition percentage against the extract concentration. Tests were carried out in triplicate, and results were averaged. The chemicals used in the study were purchased from Sigma–Aldrich.

2.6. Statistical analysis

Measurements of antioxidant activity were analyzed by a one-factor completely randomized analysis of variance (ANOVA) that compared the major chemotypes. For significant values, means were separated by the least significant difference (LSD) at $P \leqslant 0.05$, using PC SAS 8.2 (SAS Institute, Cary, NC). Pearson correlation coefficients between yields (%) and chemical composition (%) of the essential oils in the 92 plants were also analyzed using SAS 8.2.

3. Results and discussion

3.1. Yields of essential oils

The average yield of leaf essential oils in 92 *C. osmophloeum* trees for steam distillation was 0.54%, and ranged from 0.09% (plant No. 97) to 2.65% (plant No. 9) (Table 1). Yields of essential oil-bearing plants are strongly influenced by environmental and genetic factors (Sangwan, Farooqi, Sabih, & Sangwan, 2001). Because oil biosynthesis occurs in the leaves, their growth and photosynthetic capacity are important factors for oil production (Burbott & Loomis, 1967; Duriyaprapan & Britten, 1982; Srivastava & Luthra, 1994). However, we cannot exclude the possibility of oil loss after sampling. Further investigation on cultivation factors such as soil pH, planting density, harvesting time, propagation method, and year trials will be necessary before potential cutting clones can be used for efficient essential oil production. Nevertheless, these results provide important information on resource material for researchers interested in the genetic and physiological aspects of the essential oil of indigenous *C. osmophloeum* plants. These cutting clones in the clonal orchard may possess great enough variations for genetic studies.

3.2. Chemotypes of essential oils

Table 2 classifies five major chemotypes and the relative amounts (%) of major component in the leaf essential oils of 92 different cutting clones. The primary chemotype of

Table 1
Essential oil yields of 92 *Cinnamomum osmophloeum* plants

Plant No.	Content (%)	Plant No.	Content (%)	Plant No.	Content (%)
1	0.43 ± 0.04	34	1.00 ± 0.38	71	0.91 ± 0.17
2	1.23 ± 0.17	35	0.40 ± 0.08	72	1.16 ± 0.11
3	1.31 ± 0.33	36	0.09 ± 0.01	73	0.61 ± 0.01
4	1.53 ± 0.11	37	0.46 ± 0.16	75	0.57 ± 0.76
5	0.37 ± 0.05	38	0.73 ± 0.03	76	1.36 ± 0.50
6	1.37 ± 0.60	40	0.78 ± 0.36	78	0.82 ± 0.17
7	2.31 ± 0.39	41	1.29 ± 0.49	79	1.04 ± 0.26
8	1.48 ± 0.13	42	0.22 ± 0.06	80	0.77 ± 0.09
9	2.65 ± 0.90	43	0.74 ± 0.06	81	1.04 ± 0.29
10	1.17 ± 0.64	44	1.04 ± 0.36	82	0.82 ± 0.05
11	1.74 ± 0.58	49	0.33 ± 0.08	84	0.59 ± 0.21
12	1.05 ± 0.46	50	0.93 ± 0.37	85	0.09 ± 0.08
13	0.94 ± 0.04	51	0.22 ± 0.07	86	1.19 ± 0.12
14	0.78 ± 0.07	52	0.36 ± 0.08	87	1.29 ± 0.08
15	0.39 ± 0.19	53	1.22 ± 0.57	88	0.81 ± 0.08
16	1.17 ± 0.24	54	0.25 ± 0.08	89	2.19 ± 0.53
17	1.37 ± 0.38	55	1.99 ± 0.54	90	0.94 ± 0.51
18	0.64 ± 0.10	56	2.31 ± 0.15	92	1.19 ± 0.03
19	2.45 ± 0.95	57	0.13 ± 0.01	93	1.97 ± 0.78
20	0.58 ± 0.03	59	1.10 ± 0.39	94	0.68 ± 0.13
21	1.78 ± 0.53	60	0.87 ± 0.14	95	1.71 ± 0.20
22	1.69 ± 0.48	61	1.05 ± 0.34	96	0.77 ± 0.29
23	1.76 ± 0.23	62	0.38 ± 0.19	97	0.09 ± 0.04
24	1.69 ± 0.69	63	0.76 ± 0.30	98	1.34 ± 0.37
25	0.25 ± 0.05	64	1.06 ± 0.39	99	1.53 ± 0.09
26	0.52 ± 1.27	65	0.94 ± 0.44	100	0.36 ± 0.47
27	2.08 ± 0.22	66	1.04 ± 0.19	101	0.11 ± 0.03
28	1.35 ± 0.05	67	0.75 ± 0.20	102	0.82 ± 0.42
29	0.29 ± 0.06	68	1.68 ± 0.75	103	0.75 ± 0.14
31	1.24 ± 0.64	69	1.23 ± 0.60	104	1.29 ± 0.02
33	1.02 ± 0.43	70	1.19 ± 0.19		

The data are presented as mean ± standard deviation of three determinations.

Table 2
Major chemical components of the essential oils from 92 *Cinnamomum osmophloeum* plants

Compound	Constituent (%)	Plant No.
Cinnamaldehyde	53.4–95%	1, 2, 3, 4, 5, 6, 7, 9, 13, 15, 17, 18, 19, 21, 22, 23, 24, 26, 27, 28, 31, 40, 41, 43, 49, 50, 51, 56, 59, 60, 63, 64, 65, 66, 67, 71, 72, 73, 78, 82, 86, 87, 88, 90, 95, 96, 100, 102, 103, 104
linalool	73.9%	70
β-Cubebene	59.4% and 78.7%	25 and 55
Cinnamyl acetate	61.8%	44
Mixed type	<20% of each compound	8, 10, 11, 12, 14, 16, 20, 29, 33, 34, 35, 36, 37, 38, 42, 52, 53, 54, 57, 59, 61, 62, 68, 69, 75, 76, 79, 80, 81, 84, 85, 89, 92, 93, 94, 97, 98, 99, 101

the essential oils was cinnamaldehyde, ranging from 53.4% to 95%, and identified in 50 individual plants. The contents of β-cubebene in plants No. 25 and No. 55 were 59.4% and 78.7%, respectively. Linalool (73.9%) and cinnamyl acetate (61.8%) were the major compounds in plants No. 70 and No. 44, respectively. The leaf essential oils from the remaining plants were classified as mixed type because of the lack of a dominant compound, at ⩽20% for each compound. Compositions of leaf essential oil were tabulated in Table 3 for plant No. 29 containing 34 compounds (the greatest), and No. 87 containing five compounds (the least), respectively. Therefore, marked differences in the compositions of essential oils were observed among these trees from the same *Cinnamomum* species. It is well known that the quantitative composition of the essential oils of many aromatic plants is greatly influenced by the genotype and agronomic conditions, such as harvesting time, plant age and crop density (Marotti, Piccaglia, Giovanelli, Deans, & Eaglesham, 1994). In our case, no significant differences in the correlation between yields (Table 1) and the corresponding classification (Table 2) of the essential oil chemotypes were detected (data not shown). It is probable that the differences in the contents and components of the essential oils in the 92 indigenous cinnamon plants could be attributed to the result of a combination of factors such as genotype, ontogeny, light, temperature, water, and nutrients.

From a phytochemical viewpoint, the volatile oils obtained from the leaf, bark, root bark, and buds vary significantly in chemical composition. The classic primary constituents are cinnamaldehyde in the bark oil, eugenol in the leaf oil, camphor in the root-bark oil, and α-bergamotene and α-copaene in the bud essential oil (Chericoni et al., 2005). However, the main constituents and their percentages in our samples differed from those of other studies (Chang & Cheng, 2002; Cheng, Liu, Tsai, Chen, & Chang, 2004; Kordali et al., 2005; Sacchetti et al., 2004). From a business point of view, leaves seem to be a more economical and accessible source of cinnamon oil rather than isolation from the bark.

Table 3
Compounds identified in leaf essential oil of plants Nos. 29 and 87

Compound		KI	
No. 29	No. 87	No. 29	No. 87
α-Pinene	α-Pinene	1039	1039
Camphene	Camphene	1082	1082
β-Pinene	Linalool	1125	1540
δ-3-Carene	Bornyl acetate	1164	1714
Myrcene	Cinnamaldehyde	1172	2047
Phellandrene		1180	
Limonene		1212	
1,8-Cineole		1224	
E-2-Hexenal		1234	
4-Terpineol		1284	
6-Methyl-5-hepten-2-one		1346	
β-Cubebene		1383	
Linalool oxide 1		1448	
Linalool oxide 2		1476	
Linalool		1540	
Benzaldehyde		1545	
Benzyl acetate		1597	
Citral 1		1698	
Borneol		1709	
Methyl methacrylate		1725	
Citral 2		1752	
Citronellol		1761	
Z-3-hexen-1-ol		1781	
Caryophyllene oxide		1844	
Spathulenol		1991	
Cinnamaldehyde		2047	
Cinnamyl acetate		2066	
α-Phenyl allylalcohol		2090	
p-Cymene		2102	
Estragol		2119	
δ-Cadinene		2138	
Cinnamyl alcohol		2164	
Isoeugenoll		2189	
Isospathulenol		2207	

KI: Kovat's indices relative to *n*-alkanes (C_8–C_{25}).

3.3. DPPH radical-scavenging activities of the essential oils

Several methods are used to measure the antioxidant activity of a biological material. The most commonly used for their ease, speed, and sensitivity are those involving chromogen compounds of a radical nature. The presence of an antioxidant leads to the disappearance of these radical chromogens, one of the most widely used being the DPPH radical. DPPH is a free radical that can be directly acquired without preparation. The DPPH radical is scavenged by antioxidants through the donation of hydrogen, which forms the reduced compound, DPPH–H. The color changes from purple to yellow after reduction, which can be quantified by a decrease in the absorbance at 517 nm. Table 4 indicates that with 2 μg/ml of the crude extract, all major components of the essential oils in these plants expressed equivalent abilities to scavenge DPPH radicals. Yet both cinnamaldehyde and cinnamyl acetate exhibited

Table 4
Effect of the primary components of essential oils on the scavenging of DPPH radicals

Major chemical component	Scavenging of DPPH radicals (%)				
	2 μg/ml	4 μg/ml	8 μg/ml	12 μg/ml	20 μg/ml
Cinnamaldehyde	6.1 NS	18.0 a	42.3 a	62.9 a	81.9 a
Linalool	1.3 NS	9.3 b	15.3 b	17.1 c	34.4 c
β-Cubebene	2.6 NS	6.0 b	16.0 b	30.9 b	51.9 b
Cinnamyl acetate	7.1 NS	21.8 a	45.4 a	66.4 a	84.2 a

Values with different letters significantly differ ($p \leqslant 0.05$). Each value is the mean of three replicate determinations.
NS: no significant difference.

Table 5
IC_{50} values of the DPPH assay for the primary components of the essential oils of leaves of *Cinnamomum osmophloeum*

Major chemical component	IC_{50} (μg/ml)
Cinnamaldehyde	11.0
Linalool	29.7
β-Cubebene	19.3
Cinnamyl acetate	10.4

significantly higher DPPH radical-scavenging percentages than either linalool or β-cubebene at 4, 8, and 20 μg/ml of the extract. For linalool, the percentage of DPPH radicals scavenged was significantly lower than that of β-cubebene at 12 and 20 μg/ml of the extract. The higher the extract concentration was the higher was the percentage of DPPH radicals scavenged found among the major chemical components of the essential oils. The scavenging effect of the essential oils on DPPH radicals linearly increased with increasing concentration. Thus, different chemical components of the essential oils displayed variations in their DPPH radical-scavenging activity percentages. Both cinnamyl acetate and cinnamaldehyde appeared to be actively involved in the scavenging of DPPH radicals.

In recent years, increasing attention has been paid by consumers to the health and nutritional benefits of plant parts. Antioxidant properties of essential oils from many plants have also been of great interest to the food processing industry, since their possible use as natural additives has emerged from a growing tendency to replace synthetic antioxidants with natural ones (Sokmen et al., 2004). Owing to its excellent protective features exhibited in antioxidant activity tests, the essential oil from the leaves of individual of *C. osmophloeum* plants can be used as new valuable ingredients for food and/or nutriceutical and cosmeceutical applications for promoting health. The data also suggest that this essential oil may be a candidate for flavouring with functional properties in food or cosmetic products, with particular relevance for supplements in which free radicals are closely implicated. However, the efficacy and safety of this oil need to be further investigated if it is to be used as a natural agent.

The various major components of the essential oil were subjected to screening for their antioxidant activity by DPPH free-radical scavenging. The IC_{50} values for the four chemotypes were 10.4 (cinnamyl acetate type), 11.0 (cinnamaldehyde), 19.3 (β-cubebene), and 29.7 (linalool type) μg/ml (Table 5). A lower IC_{50} value indicates greater antioxidant activity. The free radical-scavenging activities of both cinnamyl acetate and cinnamaldehyde chemotypes were superior to those of the linalool and β-cubebene chemotypes. Therefore, cinnamyl acetate and cinnamaldehyde are effective radical scavengers. As a positive control, the amount of vitamin E (as reference compound) in the DPPH assay was 61.1 μg/ml.

Results in Tables 4 and 5 illustrate the effect of the essential oil from leaves of *C. osmophloeum* on DPPH. Different chemotypes of the essential oil may prevent oxidative damage by upregulating their antioxidant contents. Among the four major components, cinnamyl acetate exhibited the lowest IC_{50} values. Antioxidant defences protect plants against oxidative bursts, but they are not 100% efficient, and so free radical damage must constantly be repaired. The oxidative damage caused by free radicals to lipids, proteins, and nucleic acids is overwhelming. These free radicals can inactivate various Calvin–Benson cycle enzymes and are involved in oxidative systems, marking proteins for degradation (Chaudiere & Illious, 1999). The toxic radicals can be removed through the mobilisation of antioxidant reserves, which react both enzymatically and chemically with toxic molecular species and their products. These protective effects are considered, in large part, to be related to the various antioxidants they contained. Descriptions of the antioxidant potentials of essential oil often refer to concepts such as synergism, antagonism, and activity. For instance, DPPH-scavenging activity is one of various mechanisms that contribute to the overall activity, thereby creating a synergistic effect. Hence, the ability and capacity of other antioxidants, such as prophyrins, carotenoids, polyphenols, flavonoids, and their reductive powers, scavenging superoxide anion percentages, and conjugated diene inhibition percentages (Dinis, Madeira, & Almeida, 1994; Pulido, Bravo, & Calixto, 2000), remain to be detailed in further studies.

In conclusion, plants possess different antioxidant properties, depending on the contents of antioxidant molecules, which are, in turn, strongly affected by the specific plant genotypes. Essential oils are, from a chemical point of view, quite complex mixtures composed of several dozen components, and these complexes make it difficult to easily explain their activity pattern. *C. osmophloeum* possesses a high amount (>50%) of cinnamyl acetate, cinnamaldehyde, β-cubebene, and linalool and exhibits clear DPPH-scavenging activity. Different plants exhibited different abilities to scavenge DPPH radicals. Taking into account the high DPPH-scavenging activity, individual plants containing high levels of the above-mentioned components as specific flavour compounds, may be considered as good phenotypes and can be selected for propagation to study their genetic stability. Large variations in the essential oil contents and chemical compounds were apparent among the

tree populations, so we are currently using the molecular marker (i.e. inter simple sequence repeat – ISSR) to associate with *C. osmophloeum* plants containing the most cinnamaldehyde in this clonal orchard. Using ISSR markers as a selection tool for cinnamaldehyde production is a process that is currently being carried out. These findings are also informative for germplasm conservation, propagation, diversity, and production of *C. osmophloeum.*

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