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Chemical Composition of the Essential Oils from Cell Culture of *Dendrobium parishii* Rchb. f.

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ABSTRACT

This study identified the chemical composition of essential oils produced by cell culture of Dendrobium parishii Rchb. f. The cell cultures were established on Vacin and Went (VW) medium without plant growth regulator. The chemical compounds of essential oils were determined by Gas Chromatography/Mass Spectrometry (GC/MS) coupled with Head Space Solid Phase Micro-extraction (HS-SPME). Twenty-nine compounds of essential oils were identified. The main constituents were α-panasinsen (16.34%), hexadecanoic acid, ethyl ester (10.48%) and β-selinene (9.08%). Sesquiterpenes were the major constituents of the essential oils synthesized by cell culture of D. parishii Rchb. f. This research is the first to report on the composition of the essential oils obtained from this plant by cell culture as shown here, a promising technique for larger-scale and faster production of these essential oils. The chemical compounds identified may offer the potential for medicinal and cosmetic use.

Keywords: Cell culture, Chemical composition, *Dendrobium parishii* Rchb. f., essential oils

INTRODUCTION

essential oils are volatile, strongly odorous compounds found only in some specific plants. They are composed of a mixture of hydrocarbons and oxygenated compounds. The main constituents are terpenes and aromatic compounds. Essential oils help protect plants from bacteria, fungi and viruses. Reports on biological activities of essential oils included cytotoxic, antimutagenic and anticarcinogenic activity (Bakkali et al., 2008; evans, 2009). Orchids, like other plants, can produce a large number of phytochemicals, but only a few have been investigated for their biological functions. Orchid phytochemicals are generally categorized as alkaloids, flavonoids, carotenoids, anthrocyanins and sterols.

Dendrobium parishii Rchb. f. (Orchidaceae) is a sympodial epiphytic orchid. Endemic to Thailand, it grows easily and, with its beautiful flower (fuzzy lip with dark purple center) and fragrant scent, is a popular ornamental flower (Wood, 2006). It grows slowly through the division of pseudo bulbs. Given *D. parishii* Rchb.f can be grown easily, along with its popularity and ready availability, identifying the chemical constituents of the essential oils of this plant may offer the exploitation of wild plants for medicinal and cosmetic uses. Information in the literature about the chemical composition of the essential oils of this orchid is limited to one other paper (Julsrigival et al., 2013). But their study used the intact plant, rather than the tissue culture technique used here.

To isolate the essential oils of *D. parishii* Rchb.f, suspension culture technology was used. Normally, essential oils are produced specifically in the flower parts of orchids. As orchids flower only once a year, this can be a limiting factor. However, using cell culture technique can solve this problem. Others have investigated plant cell culture technologies as tools for large-scale production of plant masses and essential oils. Several essential oils were produced by cell culture from *Pimpinella anisum* (Ernst, 1989), Melissa offinalis (Gbolade and Lockwood, 1992) and *Thymus vulgaris* (Tamura et al., 1993). *Dendrobium* species are known for producing a variety of secondary metabolites such as alkaloids, bibenzyls, fluorenones, phenanthrenes and sesquiterpenes (Hossain, 2011). Several studies have reported on the chemical compounds isolated from orchid cell cultures such as polysaccharides from *D. huoshanense* (Wei et al., 2007; Zha et al., 2007; Wang et al., 2009), but fewer have reported on the volatile compounds.

Having isolated the essential oils, this study used Gas Chromatography/Mass Spectrometry (GC/MS) coupled with Head Space Solid Phase Micro-extraction (HS-SPME) to identify the chemical constituents. This methodology is fast and simple and provides for solvent-less extraction.

This study is the first in the literature to identify the chemical constituents of the essential oils in plant cell cultures of *D. parishii* Rchb.f. using a tissue culture technique, and in so doing will investigate a possible tool for producing plant masses and extracting essential oils on a large scale.

MATERIALS AND METHODS

Tissue culture

Plants were gathered from a cultivated farm in Chiang Mai, Thailand. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Chiang Mai University, Thailand, with code number 023109. Protocorms were induced from seeds of *D. parishii* Rchb. f. on Vacin and Went (Vacin and Went, 1949) medium containing 2% (w/v) sucrose and the pH was adjusted to 5.0 with HCl or NaOH 0.1 N before gelling it with 0.8% agar (Serva, Heidelberg, Germany). The medium was sterilized in an autoclave at 121°C for 15 min. Two hundred and fifty ml glass bottles (Duran, Mainz, Germany) were used for the culture vessel. The glass bottles were filled with 50 ml medium and all cultures were incubated under 12 h light per day at 25±2°C. After six months, the proto-

corms (approximately 6g) were transferred to a new liquid medium with the same composition as before, except without the agar, in 24 h continuous light at 25±2°C on an orbital shaker (120 rpm). Suspension cultures were sub-cultured every 28 days. The cells were harvested on days 0, 7, 14, 21, 28 of culture, weighed for growth, lyophilized and stored at -20°C. All reagents and solvents were either GC or analytical grade. *n*-Alkane solution (C8-C20) was purchased from Sigma-Aldrich Co.

Head space solid phase micro-extraction (HS-SPME)

Essential oils of cultured orchid suspension samples were extracted by HS-SPMe and analyzed using gas chromatography/mass spectrometry. One hundred milligrams of protocorms were placed in 20 ml screw-capped glass vials (National Scientific, Rockwood, TN, USA). The vials were tightly capped with a PTFE-silicon septum. An AOC 5000 Combi PAL SPME holder (CTC Analytics AG., Switzerland) with a 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber assembly (Supelco, Bellefonte, PA, USA) was used. The fiber was preconditioned according to manufacturer specification. Vials containing samples were pre-equilibrated and heated at 80°C for 30 min on a heating platform with agitation at 500 rpm.

Gas chromatography/mass spectrometry analysis

The GC-MS used was a Shimudzu GCMS-QP 2010 Plus system (Shimudzu, Kyoto, Japan). The column was a 30 m × 0.25 mm DB-5 MS capillary with 0.25 µm film thickness (J & W Scientific, Folsom, CA, USA). The carrier gas was helium at a flow rate of 1 ml min^{-1}. Samples were injected by placing the SPMe fiber in the GC inlet for 2 min. The starting temperature was 60°C, then raised to 200°C at a rate of 5°C min^{-1} and held at 200°C for 10 min. The mass spectrometer was operated in the electron impact mode with ion source temperature of 200°C, using an ionization voltage of 70 eV. The mass range was 40-400 amu. Volatile compounds were identified by comparing the obtained mass spectra of relevant chromatographic peaks with spectra of the WILEY 7 library and Kovats retention indices with other published mass spectra (Adam, 2007). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkane under the same conditions of analysis.

Statistical analysis

Cell growth measurements were carried out in triplicate and the results were expressed as mean ± standard deviation (SD) and the statistical significance ($p<0.05$) of differences among means were assessed by one-way analysis of variance (ANOVA).

RESULTS

Suspension cell cultures of *D. parishii* Rchb. f. were established and evaluated on cell growth. The cell growth was measured in terms of both the fresh

and dry weights of the suspension cells. After 28 days, fresh and dry weights were 12.3 and 1.1 g. Compared to the initiation culture, suspension cells were grown and increased two and three fold (wet weight and dry weight, respectively) (Figure 1).

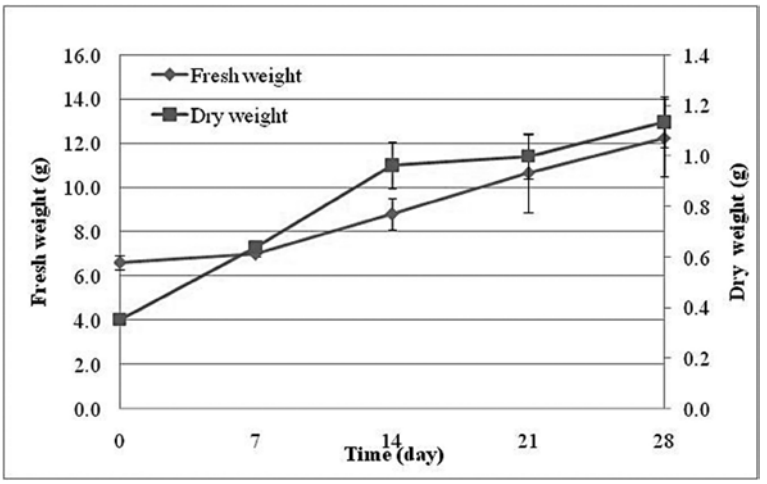

Figure 1. Growth curves of cell culture of *D. parishii* Rchb. f. on VW medium and growth period 28 days.

The volatiles obtained from the cell cultures of *D. parishii* Rchb. f. are presented in Table 1.

Table 1. Chemical composition of essential oils from cell culture of *D. parishii* Rchb. f. on VW medium and growth period 28 days.

No.	RT	Name	KI	Chemical groups	Formula	MW	RC (%)
1	2.866	Hexanal	802.28	Aldehyde	$C_6H_{12}O$	100	2.14
2	3.513	*2-trans*-Hexenal	857.53	Aldehyde	$C_6H_{10}O$	98	2.58
3	3.698	Hexanol	871.46	Alcohol	$C_6H_{14}O$	102	1.40
4	5.667	*1*-Octen-*3*-ol	984.59	Alcohol	$C_8H_{16}O$	128	0.99
5	5.855	*2*-Pentylfuran	993.17	Miscellaneous	$C_9H_{14}O$	138	0.86
6	6.885	*3*-ethyl-*2*-methyl-*1,3*-hexadiene	1040.68	Miscellaneous	C_9H_{16}	124	1.25
7	7.494	*2-trans*-Octenal	1066.02	Aldehyde	$C_8H_{14}O$	126	0.86
8	8.517	Linalool	1105.27	Monoterpene	$C_{10}H_{18}O$	154	3.20
9	9.859	Camphor	1159.18	Monoterpene	$C_{10}H_{16}O$	152	0.99
10	9.923	(*2-trans,6-cis*)-Nonadienal	1161.57	Aldehyde	$C_9H_{14}O$	138	2.72
11	10.097	*2-trans*-Nonenal	1167.97	Aldehyde	$C_9H_{16}O$	140	2.64
12	15.102	Benzenepropanoic acid, ethyl ester	1357.56	ester	$C_{11}H_{14}O_2$	178	2.36
13	16.045	*beta*-Patchoulene	1392.50	Sesquiterpene	$C_{15}H_{24}$	204	1.72
14	17.291	*alpha*-Guaiene	1443.51	Sesquiterpene	$C_{15}H_{24}$	204	2.36

No.	RT	Name	KI	Chemical groups	Formula	MW	RC (%)
15	17.594	Dihydropseudoionone	1455.75	Monoterpene	$C_{13}H_{22}O$	194	1.65
16	17.835	*alpha*-Humulene	1465.33	Sesquiterpene	$C_{15}H_{24}$	204	0.86
17	18.191	*gamma*-Gurjunene	1479.25	Sesquiterpene	$C_{15}H_{24}$	204	6.13
18	18.538	*gamma*-Selinene	1492.56	Sesquiterpene	$C_{15}H_{24}$	204	2.63
19	18.672	*beta*-Selinene	1497.63	Sesquiterpene	$C_{15}H_{24}$	204	9.08
20	19.422	*alpha*-Panasinsen	1530.32	Sesquiterpene	$C_{15}H_{24}$	204	16.34
22	21.063	Dodecanoic acid, ethyl ester	1598.60	ester	$C_{14}H_{28}O_2$	228	1.05
23	21.538	Tetradecanal	1620.40	Aldehyde	$C_{14}H_{28}O$	212	2.40
24	23.817	Pentadecanal	1722.90	Aldehyde	$C_{15}H_{30}O$	226	1.99
25	25.464	Tetradecanoic acid, ethyl ester	1799.24	ester	$C_{16}H_{32}O_2$	256	5.19
26	26.459	Hexahydrofarnesyl acetone	1849.02	Sesquiterpene	$C_{18}H_{36}O$	268	1.50
27	27.511	Pentadecanoic acid, ethyl ester	1899.76	ester	$C_{17}H_{34}O_2$	270	8.25
28	29.152	Sandaracopimaradiene	1977.67	Diterpene	$C_{20}H_{32}$	272	3.46
29	29.639	Hexadecanoic acid, ethyl ester	1999.96	ester	$C_{18}H_{36}O_2$	284	10.48

Note: RT, retention time (min); RC, relative content (%); KI, Kovats retention indices calculated against *n*-alkane standards (C8-C20).

Twenty-nine compounds were identified. The main components were α-panasinsen (16.3%), hexadecanoic acid, ethyl ester (10.5%) and β-selinene (9.1%). The essential oils were mainly composed of sesquiterpene at 43.5% (Figure 2).

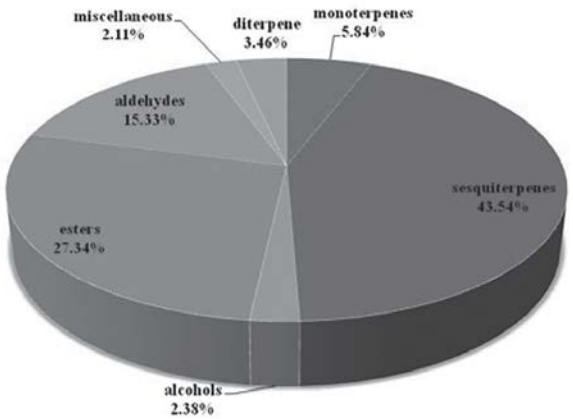

Figure 2. Chemical groups of cell culture of *D. parishii* Rchb. f.

DISCUSSION

The main volatile compounds analyzed from cell cultures of *D. parishii* Rchb. f. were a group of sesquiterpenes. In contrast, our preliminary study analyzing essential oil from the flower parts of this orchid found primarily ketones, with only trace amounts of sesquiterpenes (Julsrigival et al., 2013).

Compared to the other species of Dendrobium genus; methyl ketone and 2-alkyl acetate were reported as the major compounds of the volatile components of *D. superbum* Rchb. f. (Flath and Ohinata, 1982). According to Kaiser (Kaiser, 1993), linalool is an important compound in the essential oils obtained from cell cultures of *D. parishii* Rchb. f. and were predominantly found in the flower parts of *D. anosmum, D. antennatum, D. beckleri, D. brymerianum, D. carniferum, D. lichenastrum, D. moniliforme, D. monophyllum, D. virgineum* and *D. williamsonii*. The results obtained from this study revealed that cell cultures of *D. parishii* Rchb. f. are able to produce some essential oils compounds such as monoterpenes and sesquiterpenes, which are important chemical compounds having highly valuable properties for cosmetic, perfume, pharmaceutical and sanitary production.

This plant cell culture is a good technique for investigating chemical analysis in in vitro study. In addition, it can increase the quality and quantity of essential oils produced, especially the number of chemical compounds, i.e. sesquiterpene groups can be synthesized from eight compounds compared with six compounds in the intact plant (Julsrigival et al., 2013). This is because substantially more plant cells can be regenerated in suitable culture medium and within less time than in vivo growth. Using this method, suspension cells can be grown and increased two and three fold (wet weight and dry weight, respectively) within a short period of time (28 days). This provides a useful method for large-scale production of plant cells, producing more essential oils and plant mass than intact plant techniques.

This study is the first report on the composition of the essential oils obtained from cell cultures of *D. parishii* Rchb. f. using a tissue culture technique.

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