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The contents of bibenzyl derivatives, flavonoids and a phenanthrene in selected *Dendrobium* spp. and the correlation with their antioxidant activity

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ABSTRACT: *Dendrobium* spp. (the *Orchidaceae* family) is widely distributed in Asian countries. The fresh or dried stems of *Dendrobium* spp. have long been used in traditional Chinese medicine for their tonic and antipyretic properties. Bibenzyl derivatives (moscatilin, gigantol, crepidatin, and chrysotoxin), flavonoids (eriodictyol and homoeriodictyol) and phenanthrene lusianthridin are the major active compounds in the stems of *Dendrobium* spp. Most of these compounds have been recorded in these plants in Thailand; however, their concentrations have not yet been reported. Therefore, in this study, dried stems of selected *Dendrobium* spp. (33 species) in Thailand were extracted and analyzed by using high–performance liquid chromatography (HPLC). The HPLC method was performed on a reversed-phase column using a mobile phase gradient system of 20–32% acetonitrile in 1.5% acetic acid with a flow rate of 0.8 ml/min and a detection wavelength of 280 nm. The HPLC method displayed good linearity with coefficients of determination ($R^2 > 0.997$) for the seven active compounds analyzed. The variation in intra- and inter-day analysis (%RSD) was less than 0.5%. Additionally, the extracts of selected *Dendrobium* spp. were investigated for antioxidant activity by DPPH and FRAP radical scavenging assays. The results showed good activities in some species. The correlation between the seven active compounds and the antioxidant levels (IC_{50} and FRAP values) were significant in bibenzyl derivatives and homoeriodictyol as determined by the Pearson correlation. In conclusion, the developed HPLC method can be used for the determination of active compounds in *Dendrobium* spp., which is useful for further studies of the species containing the highest levels of these compounds.

KEYWORDS: *Dendrobium* spp., bibenzyl derivatives, flavonoids, phenanthrene, high-performance liquid chromatography, antioxidant activity

INTRODUCTION

Dendrobium spp. (*Orchidaceae* family) are widely distributed in Asian countries. The fresh or dried stems of different species of *Dendrobium*, especially *D. nobile* (known as 'Shi-hu' or 'Huang-cao'), have been used in traditional Chinese medicine as tonics, antipyretics and for the treatment of diabetes[1–3]. In the Ayurvedic system of medicine in India, orchids are commonly used as drugs such as 'jewanti' (*D. alpestre*)[3]. In the north and the northeastern parts of Thailand, *Dendrobium* species are grown in mountainous areas with a humid environment. Many *Dendrobium* species have been recorded in the office of the forest herbarium of Thailand; however, the content of active compounds and their bioactivities have not yet been reported.

Bibenzyl derivatives (moscatilin, gigantol, crepidatin and chrysotoxin), flavonoids (eriodictyol and homoeriodictyol) and phenanthrene lusianthridin are the major active compounds that can be found in the stems of *Dendrobium* spp.[4]. Pharmacological studies have demonstrated that moscatilin has antimutagenic activity in human colorectal cancer cells and anti-angiogenesis activity was seen *in vitro* and *in vivo* tests[5,6]. Bibenzyl and phenanthrene

compounds, such as gigantol, moscatilin and lusianthridin, exhibit anti-migratory activities and can inhibit filopodia formation in nonsmall cell lung cancer cells[7–9]. In addition, the antiplatelet aggregating activity of moscatilin, homoeriodictyol and gigantol was reported in an *in vitro* test[10]. Therefore, the analysis method for markers in *Dendrobium* spp. available in Thailand would provide information about which species contain the highest levels of bioactive markers. Implementation of high-performance liquid chromatography (HPLC) with mass spectrometry and diode array detection methods for the analysis of bioactive phenols in *Dendrobium aurantiacum* var. *denneanum*[11] and *Dendrobium* spp.[12] were reported.

In this study, the simultaneous determination of seven important markers by HPLC-UV was developed, and the levels of chemical markers from *Dendrobium* spp. and their antioxidant activities were evaluated for correlation. The results from this study will be useful for further studies in specified details.

MATERIALS AND METHODS

Chemicals and reagents

The reference compounds, bibenzyl derivatives (moscatilin, gigantol, crepidatin, chrysotoxin), flavonoids (eriodictyol and homoeriodictyol) and phenanthrene lusianthridin, were obtained from Dr. Boonchoo Sritularak in a previous study[7–9]. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Plant materials

A total of 33 samples of *Dendrobium* species were obtained from local markets in the Khon Kaen province and Bangkok, Thailand. The plant samples were selected based on their size relevant to 1- to 2-year-old cultivated plants. All specimens were identified by Dr. Boonchoo Sritularak. Reference specimens (NI-PSKKU073-106) were deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Sample preparation for HPLC analysis and antioxidant assays

The stems of *Dendrobium* species were dried at 50 °C until achieving a constant weight and then ground into a powder. Dried powder was accurately weighed to 50 mg per microcentrifuge tube, and 0.5 ml of methanol was added. Ultrasonic-assisted extraction was done for 15 min at ambient temperature conditions. Then, the extract was centrifuged at 5000 rpm for 5 min. The supernatant was collected into a new tube. The extraction was repeated such that the powder was extracted three times. The combined supernatant was then evaporated to dryness at room temperature and redissolved in 1 ml methanol for HPLC analysis and antioxidant assays.

Chromatographic conditions

The HPLC system was performed on a SHIMADZU i-Series. A RP-18 column (LichroCART, 250 mm × 4 mm, 5 μm particle size, Merck, Germany) was used. The mobile phase system consisted of a gradient elution starting at 20% acetonitrile in 1.5% aqueous acetic acid, which gradually increased to 32% acetonitrile in 30 min, and was held at 32% acetonitrile for 30 min. The flow rate was 0.8 and 1 ml/min for 0–30 min and 30–60 min of runtime, respectively. The system was set at a temperature of 20 °C. All solvents were filtered with a Whatman 0.45 μm nylon filter prior to use, and the detection wavelength was set at 280 nm. The sample solutions were filtered and injected (20 μl per injection) by an autosampler. The peak area and chromatograms of each sample were recorded and analyzed.

Method validation

A series of standard solutions were prepared by mixing moscatilin, gigantol, crepidatin, chrysotoxin, eriodictyol, homoeriodictyol and lusianthridin to obtain solutions with final concentrations of 100.00, 50.00, 25.00, 12.50, 6.25, 3.13, 1.56, and 0.78 μg/ml for all compounds. Intraday precision was evaluated on the same day as the calibration curve by injecting each concentration of standard solution in triplicate. The linearity of each standard curve was established by plotting the peak area versus the concentration. Interday precision was determined over 3 days by repeating triplicate injections (20 μl per injection) for each concentration of standard solutions each day. Intraday and interday precision were reported as relative standard deviation (%RSD). The correlation and the regression equations were calculated in MICROSOFT EXCEL 2010. The limits of detection (LOD) and limits of quantification (LOQ) were determined by calculating the average baseline noise with a signal 3 and 10 times higher than that of the baseline noise, respectively.

The accuracy was evaluated by means of recovery studies. *Dendrobium* spp. sample solutions were spiked with known amounts of the mixed standards at three different concentration levels (25%, 50%, and 100% over the range of compound contents in *Dendrobium* spp. samples). Then, the spiked and unspiked samples were analyzed and the percentage of recovery was reported, which was calculated by comparing the added amount and the determined amount of each reference.

DPPH radical-scavenging activity assay

Dendrobium spp. extracts (33 species) were diluted by methanol to several concentrations: 0.31, 0.63, 1.25, 2.5, 5, and 10 mg dry wt./ml. Seven standard solutions were diluted to concentrations of 1.25, 2.5, 5, 10, 25, 50, 100, 200, 500, and 1,000 μg/ml in methanol. The reaction mixtures in 96-well plates consisted of sample (100 μl) and DPPH (100 μl, 0.2 mM), which were both freshly prepared by dissolution in methanol. The mixture was kept in dark conditions for 30 min at room temperature. Then, the absorbance was measured at 495 nm against a blank (methanol). All determinations were performed in triplicate. The antioxidant activity was analyzed according to an established DPPH assay protocol with some modifications [13,14]. The results were expressed as IC_{50} values, which are the concentration required to affect the scavenging of 50% of DPPH radicals. The IC_{50} values were calculated from the relationship curve of scavenging activities (%) versus concentrations of the respective sample.

Ferric reducing antioxidant power (FRAP) assay

Dendrobium spp. extracts (33 species) were diluted by methanol to concentrations of 0.31, 0.63, 1.25, 2.50, 5.00, 6.25, and 12.5 mg dry wt./ml. Seven standard solutions were diluted to concentrations of 2.5, 5, 10, 25, 50, and 100 μg/ml in methanol. The working FRAP reagent was freshly prepared and used on the same day by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM hydrochloric acid and 20 mM ferric chloride $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1 (v/v/v). The working FRAP reagent (270 μl) and sample solutions (30 μl) were mixed in 96-well plates and kept at 37 °C for 30 min. The absorbance was measured at 595 nm. A standard curve was performed using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) at concentrations of 10, 15.6, 25, 31.25, 50, and 62.5 μg/ml. All determinations were performed in triplicate. The results were analyzed by using a standard curve and expressed as the FRAP value (mg Trolox/g dry weight of sample) [13].

Table 1 Regression equation, correlation coefficients, limit of quantification (LOQ) and limit of detection (LOD) for standards determined by HPLC data analysis[†]

Cmp	Range	Regression equation	R^2	LOD[*]	LOQ[*]
1	0.78–25	$y = 70314x + 514$	0.9999	0.14	0.48
2	0.78–25	$y = 63321x + 2242$	0.9998	0.16	0.53
3	6.25–100	$y = 8493x + 4134$	0.9999	1.32	4.40
4	0.78–25	$y = 97306x - 12718$	0.9976	0.10	0.32
5	1.56–50	$y = 18508x + 13981$	0.9993	0.41	1.35
6	6.25–100	$y = 10943x - 29578$	0.9990	1.57	5.23
7	6.25–100	$y = 11987x - 40232$	0.9982	1.51	5.03

[†] Cmp = compound; Range = linearity range (μg/ml);
[*] measured in μg/ml.

Statistical analysis

The results of the HPLC and antioxidant assays are presented as the mean ± standard deviation of triplicate independent experiments. The Pearson correlation was used to analyze the relationship of the test results. The significance levels of $p < 0.05$ or $p < 0.01$ were considered to indicate significance. All statistical analyses were performed using the SPSS Statistics software version 17.0.

RESULTS

To determine the contents of bibenzyl derivatives (moscatilin, gigantol, crepidatin, chrysotoxin), flavonoids (eriodictyol and homoeriodictyol) and phenanthrene lusianthridin in *Dendrobium* species, we developed an assay using the HPLC-UV method with optimum conditions for quantitation. The optimum condition for the determination of all seven standard compounds was using a gradient elution which started at 20% acetonitrile in 1.5% aqueous acetic acid and gradually increased to 32% acetonitrile over 30 min. The column was then eluted with 32% acetonitrile for 30 min. The flow rates were 0.8 and 1 ml/min for 0-30 min and 30–60 min of runtime, respectively. The HPLC chromatograms of standards are shown in Fig. 1(A). The retention times were 19.0, 28.2, 31.6, 32.8, 34.1, 42.8, and 45.9 min for eriodictyol (1), homoeriodictyol (2), moscatilin (3), lusianthridin (4), gigantol (5), chrysotoxin (6), and crepidatin (7), respectively. Linearity was established by constructing a calibration curve for each marker compound. The regression equation shows good correlation coefficients ($R^2 > 0.997$) in each established linearity range of the seven standard compounds (Table 1). Moreover,

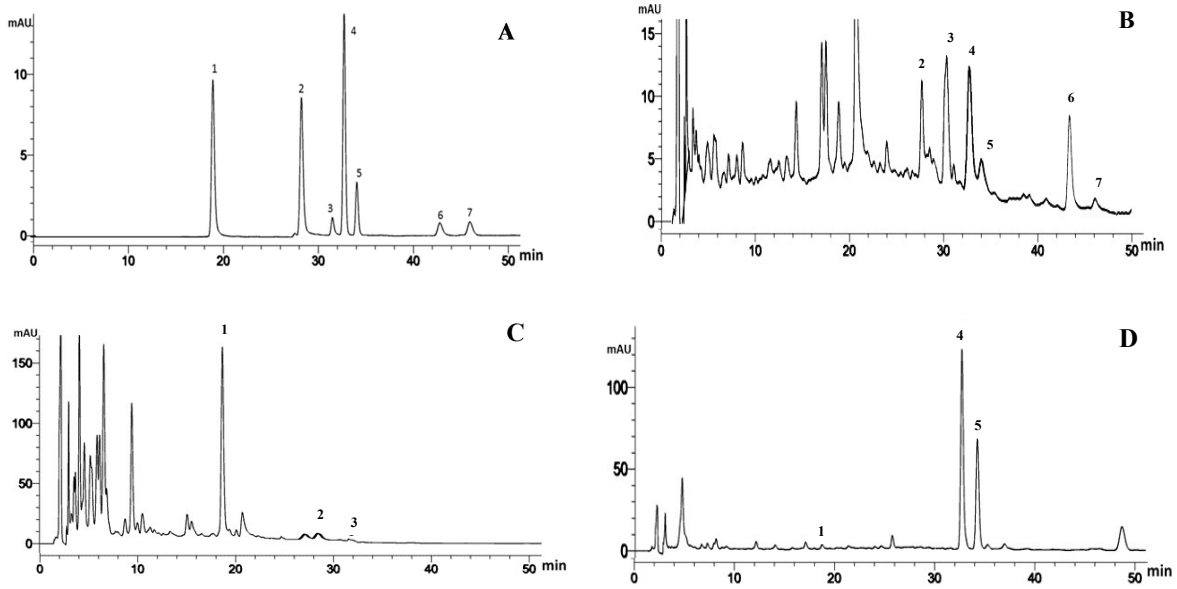

Fig. 1 HPLC Chromatograms (A) Standard compounds at a concentration of 3.13 μg/ml of (1) eriodictyol; (2) homoeriodictyol; (3) moscatilin; (4) lusianthridin; (5) gigantol; (6) chrysotoxin, and (7) crepidatin. (B) The methanol extract from the stem of *D. fimbriatum*; (C) *D. ellipsophyllum*, and (D) *D. venustum*.

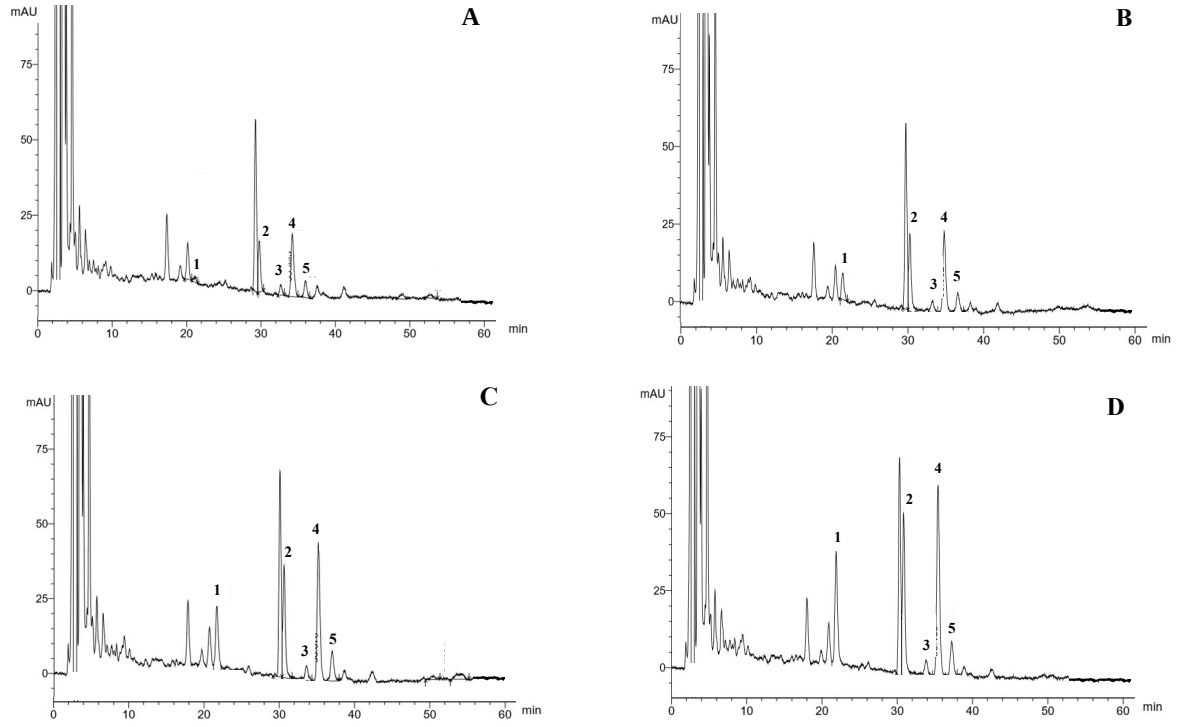

Fig. 2 A representative chromatogram of the recovery test (A) of the unspiked *D. palpebrae* methanolic extract and (B) the spiked samples of *D. palpebrae* methanolic extract at the concentration of 5 μg/ml, (C) 10 μg/ml, and (D) 20μg/ml with (1) eriodictyol; (2) homoeriodictyol; (3) moscatilin; (4) lusianthridin; and (5) gigantol.

Table 2 Recovery of standard compounds in *Dendrobium* spp. samples.†

Compound	Spiked (μg/ml)	Recovery (%)	RSD (%)
1	5	102.46	0.84
	10	90.49	1.47
	20	91.03	2.80
2	5	101.19	1.48
	10	90.93	2.29
	20	90.47	0.42
3	5	103.26	1.11
	10	95.19	1.53
	20	97.76	4.15
4	5	90.55	0.17
	10	90.57	0.56
	20	89.38	6.97
5	5	96.91	1.97
	10	91.49	2.36
	20	92.99	1.75
6	10	105.83	0.74
	20	95.33	2.32
	50	99.82	1.95
7	10	99.62	2.35
	20	90.69	4.79
	50	91.95	2.71

† Spiked = spiked concentration.

Table 3 IC_{50} (mg/ml) values determined by the DPPH assay and the antioxidant activity of seven compounds determined by the FRAP assay (mg Trolox/g dry wt.).

Compound	IC_{50}	FRAP value
1	$18.16 \pm 0.15 \times 10^{-3}$	1066.46 ± 24.51
2	$583.88 \pm 9.40 \times 10^{-3}$	857.27 ± 20.33
3	$23.23 \pm 1.21 \times 10^{-3}$	2923.58 ± 163.23
4	$21.40 \pm 0.61 \times 10^{-3}$	1071.46 ± 46.58
5	$10.75 \pm 0.16 \times 10^{-3}$	2516.63 ± 162.15
6	$21.68 \pm 0.27 \times 10^{-3}$	1248.04 ± 62.09
7	$22.45 \pm 0.90 \times 10^{-3}$	1258.04 ± 46.57
Trolox	$16.99 \pm 0.47 \times 10^{-3}$	–

the limits of detection (LOD) and quantification (LOQ), which were respectively calculated as 3 and 10 times the signal-to-noise ratio of the smallest peak detected in chromatograms, are shown in Table 1. The intraday and interday assay precision values were analyzed by using the variation in measurement of the seven marker compounds. The relative standard deviations (%RSD) of both intraday and interday were less than 5% (data not shown). The amount of each standard added was 5, 10, and 20 μg/ml. The standard compound recovery of spiked *Dendrobium* extract was tested to establish accuracy. After the extract was spiked with different concentrations of the seven standard compounds, the concentration was determined and calculated as a percentage of recovery, which presented good accuracy within 80–120% (Table 2). For example, the unspiked chromatogram of *palpebrae* methanolic extract is shown in Fig. 2(A). The spiked samples with (1) eriodictyol, (2) homoeriodictyol, (3) moscatilin, (4) lusianthridin, and (5) gigantol at concentrations of 5, 10, and 20 μg/ml are shown in Fig. 2(B,C,D). respectively. The contents of the bibenzyl derivatives moscatilin (3), gigantol (5), crepidatin (6), and chrysotoxin (7); the

Table 4 IC_{50} (mg/ml), FRAP value (mg Trolox/g dry wt.) of various *Dendrobium* spp. extracts determined by the DPPH and FRAP assays, respectively.

Name	IC_{50}	FRAP value
D. albosanguineum	0.93 ± 0.06	10.71 ± 0.25
D. anosmum	5.84 ± 0.27	3.15 ± 0.08
D. bellatulum	2.85 ± 0.12	11.80 ± 0.38
D. capilipes	1.78 ± 0.11	11.51 ± 0.68
D. christyanum	2.59 ± 0.20	7.75 ± 0.40
D. chrysotoxum	3.34 ± 0.27	6.92 ± 0.39
D. cretaceum	3.34 ± 0.07	7.66 ± 0.75
D. crumenatum	3.95 ± 0.14	4.05 ± 0.26
D. delacourii	1.65 ± 0.17	10.05 ± 0.52
D. draconis	1.82 ± 0.09	5.86 ± 0.22
D. ellipsophyllum	0.37 ± 0.03	25.96 ± 0.84
D. falconeri	1.19 ± 0.18	11.15 ± 0.74
D. fimbriatum	0.25 ± 0.01	53.02 ± 1.58
D. findlayanum	1.28 ± 0.07	10.70 ± 0.95
D. formosum	4.16 ± 0.02	5.16 ± 0.31
D. friedericksianum	0.78 ± 0.00	19.62 ± 1.07
D. infundibulum	2.39 ± 0.02	7.56 ± 0.73
D. kontumense	2.68 ± 0.23	10.16 ± 0.42
D. lindleyi	0.53 ± 0.49	9.06 ± 0.29
D. moschatum	9.20 ± 0.10	2.66 ± 0.20
D. ochreatum	0.83 ± 0.06	10.94 ± 0.81
D. pachyglossum	1.10 ± 0.12	12.14 ± 0.56
D. palpebrae	0.88 ± 0.01	16.01 ± 0.77
D. parishii	0.36 ± 0.01	14.82 ± 0.25
D. primulinum	0.88 ± 0.02	23.39 ± 1.11
D. pulchellum	4.92 ± 0.06	6.01 ± 0.25
D. secundum	4.46 ± 0.23	5.51 ± 0.35
D. secundum var. *alba*	3.15 ± 0.10	5.91 ± 0.25
D. signatum	1.16 ± 0.23	9.55 ± 0.29
D. soabriligue	2.59 ± 0.09	11.17 ± 0.62
D. thyrsiflorum	2.26 ± 0.04	8.25 ± 0.73
D. tortile	0.95 ± 0.02	16.34 ± 0.62
D. venustum	4.25 ± 0.02	26.02 ± 1.14

Table 5 Contents of total bibenzyl derivatives (moscatilin, gigantol, chrysotoxin, crepidatin), total flavonoids (eriodictyol, homoeriodictyol) and phenanthrene lusianthridin in stems from thirty-three *Dendrobium* spp.

Dendrobium spp.	Content (mg/g dry wt.)								
	Mos.(3)	Gig.(5)	Chr.(6)	Cre.(7)	Bibenzyl	Eri.(1)	Hom.(2)	Flavonoids	Lus.(4)
albosanguineum	0.96 ± 0.02	0.14 ± 0.00	1.13 ± 0.02	–	2.23 ± 0.05	–	–	–	–
anosmum	0.24 ± 0.00	–	–	–	0.24 ± 0.00	0.19 ± 0.00	–	0.19 ± 0.00	–
bellatulum	0.11 ± 0.01	0.21 ± 0.01	–	–	0.32 ± 0.02	–	–	–	0.10 ± 0.00
capilipes	–	0.67 ± 0.05	–	–	0.67 ± 0.05	0.07 ± 0.00	–	0.07 ± 0.00	–
christyanum	–	0.07 ± 0.00	–	–	0.07 ± 0.00	0.02 ± 0.00	–	0.02 ± 0.00	0.06 ± 0.00
chrysotoxum	0.34 ± 0.01	–	–	–	0.34 ± 0.01	–	0.09 ± 0.00	0.09 ± 0.00	–
cretaceum	1.09 ± 0.05	0.18 ± 0.01	–	–	1.27 ± 0.06	–	–	–	–
crumenatum	–	0.09 ± 0.00	–	–	0.09 ± 0.00	–	–	–	0.09 ± 0.00
delacourii	–	0.99 ± 0.03	–	–	0.99 ± 0.03	–	0.05 ± 0.00	0.05 ± 0.00	0.74 ± 0.03
draconis	0.14 ± 0.00	0.15 ± 0.00	–	–	0.29 ± 0.01	0.03 ± 0.00	–	0.03 ± 0.00	–
ellipsophyllum	0.17 ± 0.00	–	–	–	0.17 ± 0.00	1.86 ± 0.04	0.07 ± 0.00	1.93 ± 0.04	–
falconeri	0.13 ± 0.01	–	2.09 ± 0.14	–	2.22 ± 0.15	–	–	–	–
fimbriatum	3.57 ± 0.30	0.35 ± 0.01	3.02 ± 0.19	0.43 ± 0.01	7.36 ± 0.50	–	0.24 ± 0.01	0.24 ± 0.01	0.22 ± 0.02
findlayanum	–	–	0.53 ± 0.02	1.31 ± 0.02	1.85 ± 0.04	–	0.08 ± 0.00	0.08 ± 0.00	–
formosum	0.58 ± 0.02	0.09 ± 0.01	–	–	0.67 ± 0.03	0.02 ± 0.00	0.13 ± 0.01	0.16 ± 0.01	–
friedericksianum	–	0.07 ± 0.00	1.04 ± 0.01	0.31 ± 0.00	1.42 ± 0.02	0.20 ± 0.00	0.08 ± 0.00	0.28 ± 0.00	–
infundibulum	–	–	–	–	–	–	0.02 ± 0.00	0.02 ± 0.00	–
kontumense	–	1.04 ± 0.07	–	–	1.04 ± 0.07	0.02 ± 0.00	–	0.02 ± 0.00	–
lindleyi	–	–	–	–	–	–	–	–	0.06 ± 0.00
moschatum	–	–	–	–	–	0.06 ± 0.00	–	0.06 ± 0.00	–
ochreatum	1.22 ± 0.06	0.08 ± 0.00	–	–	1.30 ± 0.06	–	–	–	–
pachyglossum	0.75 ± 0.00	0.14 ± 0.01	–	–	0.89 ± 0.01	0.09 ± 0.00	–	0.09 ± 0.00	–
palpebrae	0.87 ± 0.01	0.45 ± 0.01	0.32 ± 0.00	0.29 ± 0.00	1.93 ± 0.02	0.27 ± 0.00	0.99 ± 0.01	1.26 ± 0.01	0.25 ± 0.01
parishii	2.36 ± 0.07	–	–	–	2.36 ± 0.07	0.10 ± 0.00	0.03 ± 0.00	0.13 ± 0.00	–
primulinum	0.99 ± 0.03	1.01 ± 0.02	–	–	2.01 ± 0.04	–	–	–	–
pulchellum	0.18 ± 0.00	0.02 ± 0.00	–	–	0.20 ± 0.00	–	–	–	–
secundum	–	–	–	–	–	–	–	–	–
secundum var. *alba*	–	–	–	–	–	–	–	–	–
signatum	–	–	0.59 ± 0.02	–	0.59 ± 0.02	0.18 ± 0.00	0.25 ± 0.00	0.43 ± 0.01	–
soabriligue	0.15 ± 0.01	0.50 ± 0.01	–	–	0.65 ± 0.02	0.03 ± 0.00	0.02 ± 0.00	0.24 ± 0.00	0.06 ± 0.00
thyrsiflorum	0.70 ± 0.03	0.28 ± 0.01	–	–	0.98 ± 0.04	–	0.28 ± 0.02	0.28 ± 0.02	0.07 ± 0.00
tortile	–	–	0.54 ± 0.02	–	0.54 ± 0.02	0.09 ± 0.00	0.09 ± 0.00	0.18 ± 0.00	–
venustum	–	4.13 ± 0.24	–	–	4.13 ± 0.24	0.04 ± 0.00	–	0.04 ± 0.00	1.29 ± 0.04

– = not detected.

Table 6 Correlation analysis of marker compounds in *Dendrobium* spp. and antioxidant activities.

		Assay		Compounds						
		IC_{50}	FRAP	1	2	3	4	5	6	7
IC_{50}	Pearson	1	−.566**	−.193	−.206*	−.315**	−.191	−.197	−.339**	−.204*
	Sig.(2-tailed)	–	.000	.056	.041	.002	.058	.051	.001	.043
	N	99	99	99	99	99	99	99	99	99
FRAP	Pearson	−.566**	1	.264**	.225*	.643**	.319**	.326**	.642**	.261**
	Sig.(2-tailed)	.000	–	.008	.025	.000	.001	.001	.000	.009
	N	99	99	99	99	99	99	99	99	99

*Correlation is significant at the 0.05 level (2-tailed); **correlation is significant at the 0.01 level (2-tailed).

flavonoids eriodictyol (1) and homoeriodictyol (2); and phenanthrene lusianthridin (4) in 33 species of *Dendrobium* are shown in Table 5. The results showed that different species of *Dendrobium* had different chemical contents and characterizations. The seven standard markers were determined in all samples, and the concentration in mg/g dry weight was calculated. The results show that *D. fimbriatum* contained the highest bibenzyl derivative content (7.36 ± 0.50 mg/g dry wt.) and the chromatogram is shown in Fig. 1(B). *D. ellipsophyllum* contained the highest flavonoid content (1.93 ± 0.04 mg/g dry wt.) and the chromatogram is shown in Fig. 1(C). The highest phenanthrene content (1.29 ± 0.04 mg/g dry wt.) was found in *D. venustum*, as seen in the chromatogram in Fig. 1(D).

From this study, 28 of the 33 *Dendrobium* spp. tested primarily contain compounds in the bibenzyl group, which indicates that the bibenzyl group can be used as a marker for *Dendrobium* spp. Our results

showed that 31 species contained at least one of seven compounds, which can be used to identify the chemical constituents of each species. However, detection of any of the seven standard compounds in *D. secundum* and *D. secundum* var. *alba* was not achieved.

The DPPH and FRAP assays are the basic methods for screening the antioxidant activity in plant samples, which are usually reported as IC_{50} values and in mg Trolox/g dry weight, respectively. Hence, both assays were used to determine the antioxidant activity of seven marker compounds in 33 species of *Dendrobium*. The results are shown in Tables 3 and 4 respectively. Consequently, a Pearson correlation was used to analyze the significant correlation between the content of each marker compound in *Dendrobium* spp. and their antioxidant activities.

In the DPPH assay, the contents of moscatilin and chrysotoxin showed a significant correlation with the antioxidant activity at the significance level $p < 0.01$, and the content of homoeriodictyol and crepidatin exhibited a significant correlation with antioxidant activity at $p < 0.05$. In the FRAP assay, the contents of eriodictyol, moscatilin, lusianthridin, gigantol, chrysotoxin and crepidatin were significantly correlated with the antioxidant activity ($p < 0.01$). The content of homoeriodictyol was also significantly correlated ($p < 0.05$), as shown in Table 6.

DISCUSSION

The developed HPLC method is useful for analyzing the seven marker compounds in *Dendrobium* spp. According to the analysis by the Pearson correlation, *Dendrobium* spp. which contained high levels of bibenzyl groups can be predicted to possess high antioxidant activities. Based on our results, antioxidant activities from the DPPH and FRAP assays of homoeriodictyol, moscatilin, crepidatin and chrysotoxin exhibit good relationships with their contents ($p < 0.05$). These results were also related to the summed bibenzyl derivative group concentration in *Dendrobium* spp. such as *D. fimbriatum*, which contains the highest amount of total bibenzyl derivatives (7.36 ± 0.50 mg/g) and exhibits the highest antioxidant activities in the DPPH and FRAP assays (IC_{50} 0.25 ± 0.01 mg/ml and 53.02 ± 1.58 mg Trolox/g dry wt., respectively). Therefore, bibenzyl contents can be used as antioxidant markers for *Dendrobium* species. In a previous study, bibenzyl groups in *D. nobile* also showed high antioxidant activity[15]. These antioxidant activities conform to the traditional Chinese medicine 'Shi Hu', which is derived from the dried or fresh stems of several *Dendrobium* species and is widely used for the treatment of various diseases associated with the metabolic disorders of reactive oxygen species in the human body, such as chronic atrophic gastritis, diabetes, skin aging, and cardiovascular disease[16].

Although flavonoids and phenanthrene have shown similarly high levels of antioxidant activity as the bibenzyl group, their contents only have a significant correlation ($p < 0.05$) in the FRAP assay, with the exception of homoeriodictyol, which showed a significant correlation in both the DPPH and FRAP assays. Hence, in further studies, other biological activity assays might be performed to ensure that key marker compounds can be used as bioactive markers for *Dendrobium* spp.

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