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Chemistry, bioactivity and quality control of *Dendrobium*, a commonly used tonic herb in traditional Chinese medicine

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Abstract The fresh or dried stems of many *Dendrobium* species are well known as one of the most expensive tonics in traditional Chinese medicine. Documented as a "superior grade" herbal medicine in the ancient text "*Shen Nong's Herbal Classic*", *Dendrobium* has been used for thousands of years and is now a popular health food worldwide. The main chemical components of *Dendrobium* are alkaloids, aromatic compounds, sesquiterpenoids and polysaccharides, with multiple biological activities, including immunomodulatory, neuroprotective and anti-tumor effects, etc. Various qualitative and quantitative methods have been developed for the quality evaluation of *Dendrobium*. In this review, the research progress since the 1930s relating to the chemistry, bioactivity and quality control of *Dendrobium* is summarized, existing problems and prospects are also discussed.

Keywords *Dendrobium* · Chemistry · Bioactivity · Quality control

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Introduction

Dendrobium, one of the largest genera in Orchidaceae, having more than 1,100 species identified, is widely distributed throughout Asia, Europe and Australia (Zhang et al. 2003a). In China, the fresh or dried stems of many *Dendrobium* species are collectively regarded as a famous herbal medicine. Listed as a "superior grade" medicinal herb in "*Shen Nong's Herbal Classic*," which is one of the earliest herbal pharmacopeia in the world, *Dendrobium* has been used in Chinese medicine for thousands of years for its traditional properties of supplementing the stomach, promoting the production of body fluids, nourishing *Yin,* and clearing heat (Deng et al. 2002).

Despite 78 species of *Dendrobium* found in China, thirty of which are currently used under the same Chinese name *Shihu*, Chinese Pharmacopoeia (2010 edition) has only two monographs of medicinal *Dendrobium* plants. One is Dendrobii Caulis (*Shihu* in Chinese), derived from *Dendrobium nobile*, *D. chrysotoxum*, *D. fimbriatum* and other related *Dendrobium* species. The other is Dendrobii Officinalis Caulis (*Tiepi shihu* in Chinese), derived from *D. officinale* (Fig. 1).

Fig. 1 The original plants of *D. nobile* (**a**), *D. chrysotoxum* (**b**), *D. fimbriatum* (**c**) and *D. officinale* (**d**)

Chemical studies on *Dendrobium* plants have been conducted since the 1930s. While alkaloids, aromatic compounds, sesquiterpenoids and polysaccharides have been identified as the main components (Chen and Guo 2001), the chemical profile varies greatly among species and samples collections (Xu et al. 2010b, 2011; Yang et al. 2006c) which in turn makes these *Dendrobium* species possess diverse bioactivities: *Dendrobium* polysaccharides exhibit immunomodulatory, antioxidant and hepatoprotective activity; the alkaloids are anti-cataract and neuroprotective, and the aromatic compounds and sesquiterpenoids exert anti-angiogenesis, anti-tumor and anti-mutagenesis effects (Ng et al. 2012).

Therefore, quality control becomes urgent for ensuring the efficacy and safety of *Dendrobium* in clinical applications. Chinese Pharmacopoeia (2010 edition) recommended only one chemical marker which seems powerless for so many *Dendrobium* species involved. Furthermore, there are multiple factors affecting the quality, not only species, but also geographic localities, harvest times and processing methods (Xu et al. 2010b). To date, many studies have attempted to develop accurate, sensitive and selective analytical methods for qualitative and quantitative evaluation of *Dendrobium* materials.

In this paper, the progress in researches of chemistry, bioactivity and quality control of *Dendrobium* plants is reviewed with some existing problems addressed and suggestions for further study are also proposed.

Chemistry

In the past 80 years, more than forty *Dendrobium* species have been investigated for their phytochemistry, with a focus on small molecules, such as alkaloids, aromatic compounds and sesquiterpenoids, while researches on biomacromolecules in *Dendrobium* species started 30 years ago. The results showed that different *Dendrobium* sources presented unique chemical profiles.

Alkaloids

Alkaloids are the first category of compounds extracted from *Dendrobium* with confirmed chemical structures. So far, five types of alkaloids with different structural skeletons have been reported from *Dendrobium*, namely sesquiterpenoids, indolizidine, pyrrolidines, phthalides and imidazoles (Table 1). As shown in Fig. 2a and Table 1, a sesquiterpenoid, also categorized as the dendrobine-type, has been found in six *Dendrobium* species. Its basic skeleton consists of one picrotoxane-type sesquiterpenoid combined with a

Table 1 Micromolecular compounds in *Dendrobium* spp.

Dendrobium species	Components	Refs.
D. aduncum	Fluorenones: chrysotoxone (A1[a]); dengibsin (A1); dengibsinin (A1) Sesquiterpenoids: aduncin (A1) Other kinds of compounds: monoaromatics; steroids	Bi et al. (2006), Gawell and Leander (1976)
D. amoenum	Bibenzyls: moscatilin (A); chrysotobibenzyl (A); batatasin III (A); 3,4′-dihydroxy-5-methoxybibenzyl (A); amoenylin (A); isoamoenylin (A); gastrochilinin (B1); gastrochilin (B1) Phenanthrenes: confusarin (A1); 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene (A1); imbricatin (A2); amoenumin (A2) Sesquiterpenoids: amotin (A1); amoenin (A1)	Majumder et al. (1999), Majumder and Bandyopadhyay (2010), Veerraju et al. (1989), Dahmén and Leander (1978)
D. amplum	Bibenzyls: gigantol (A); batatasin III (A) Phenanthrenes: 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene (A1); 2,3,7-trihydroxy-4,6-dimethoxyphenanthrene (A1); 2,3,7-trihydroxy-4,6-dimethoxy-9,10-dihydrophenanthrene (A2); 2,7-Dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene (A2); coelonin (A2); amplumthrin (A2); flavanthrin (A2)	Majumder et al. (2008)
D. anosmum	Alkaloids: dendroparine (E)	Leander and Lüning (1968)
D. aphyllum	Bibenzyls: moscatilin (A); gigantol (A); batatasin III (A); tristin (A); 3,4′,5-trihydroxybibenzyl (A); 4,4′-dihydroxy-3,5-dimethoxybibenzy (A) Phenanthrenes: moscatin (A1); 2,4,7-trihydroxy-9,10-dihydrophenanthrene (A2); lhridinusiant (A2); coelonin (A2); flavanthrin (A2) Other kinds of compounds: monoaromatics; flavonoids; lignans	Chen et al. (2008a), Shao et al. (2008), Zhang et al. (2008a)
D. aurantiacum var. *denneanum*	Bibenzyls: moscatilin (A); gigantol (A); chrysotobibenzyl (A); chrysotoxine (A); crepidatin (A) Phenanthrenes: moscatin (A1); confusarin (A1) Fluorenones: dendroflorin (A1); dengibsin (A1) Coumarins: coumarin (B) Other kinds of compounds: monoaromatics; steroids; flavonoids; terpenes	Chang et al. (2001), Yang et al. (2006b), Ying et al. (2009), Ma et al. (1998a), Zheng et al. (2000a), Zhang et al. (2005a), Yang et al. (2007c)
D. cariniferum	Bibenzyls: gigantol (A); batatasin III (A); 3,3′,5-trihydroxybibenzyl (A) Phenanthrenes: dendronone (C2) Other kinds of compounds: steroids	Liu et al. (2009), Chen et al. (2008c)

Table 1 continued

Dendrobium species	Components	Refs.
D. chrysanthum	Alkaloids: *cis*- and *trans*-dendrochrysine (C); *cis*- and *trans*-dendrochrysanines (C); hygrine (C) Bibenzyls: moscatiline (A); gigantol (A); chrysotobibenzyl (A); chrysotoxine (A); crepidatin (A) Phenanthrenes: moscatin (A1); 2,5-dihydroxy-4,9-dimethoxyphenanthrene (A1); denthyrsinin (A1); denchryside A (A1); dendrochrysanene (B3); cypripedin (C1) Fluorenones: dendroflorin (A1); denchrysan A (A1); dengibsin (A1); 3,5-dihydroxy-2,4-dimethoxy-9*H*-fluoren-9-one (A1); 3,5-dihydroxy-4-methoxy-9*H*-fluoren-9-one (A1); denchrysan B (A2) Other kinds of compounds: lignans; monoaromatics; anthraquinones; steroids	Ekevag et al. (1973), Yang et al. (2004d, 2005b, 2006a), Luning and Leander (1965), Ye et al. (2003, 2004a, b), Barlocco (2006)
D. chrysotoxum	Bibenzyls: gigantol (A); chrysotobibenzyl (A); chrysotoxine (A); batatasin III (A); erianin (A); trigonopol B (B3) Phenanthrenes: moscatin (A1); confusarin (A1); chrysotoxene (A1); 2,5-dihydroxy-4,9-dimethoxyphenanthrene (A1); 2,6-dihydroxy-5,7-dimethoxyphenanthrene (A1); 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene (A1); nudol (A1); fimbriatone (A1); 2,4,7-trihydroxy-9,10-dihydrophenanthrene (A2); erianthridin (A2); chrysotoxol A (B2); chrysotoxol B (B4); densiflorol B (C1) Fluorenones: dendroflorin (A1); chrysotoxone (A1); denchrysan A (A1); dengibsin (A1); 1,4,5-trihydroxy-7-methoxy-9*H*-fluoren-9-one (A1); 2,4,7-trihydroxy-1,5-dimethoxy-9*H*-fluoren-9-one (A1); denchrysan B (A2) Other kinds of compounds: monoaromatics; steroids; lignans; flavonoids	Li et al. (2009f, 2011), Yang et al. (2001, 2002, 2004a, b), Ma et al. (1994b, 1996, 1998b), Hu (2007), Chen et al. (2008b), Gong et al. (2006)
D. crepidatum	Alkaloids: crepidine (B1); crepidamine (B2); isocrepidamine (B2); 1-((5*S*, 6*R*, 7S, 8a*R*)-6-hydroxy-7-methyl-6-phenyl-octahydroindolizin-5-yl) propan-2-one (B2); dendrocrepine (B2); isodendrocrepine (B2) Bibenzyls: moscatilin (A); crepidatin (A) Other kinds of compounds: monoaromatics; steroids; flavonoids; terpenes	Elander et al. (1973), Hu (2007), Zhao et al. (2011), Majumder and Chatterjee (1989)
D. crystallinum	Bibenzyls: gigantol (A); batatasin III (A); 3-*O*-methylgigantol (A); 3,3′-dihydroxy-5-methoxybibenzyl (A); 3,4′-dihydroxy-5-methoxybibenzyl (A); 4,4′-dihydroxy-3,5-dimethoxybibenzy (A); 3,5′-dihydroxy-3′,4-dimethoxybibenzy (A); 4′-hydroxy-3,3′,5-trimethoxybibenzyl (A); dencryol A (B4); dencryol B (B4) Phenanthrenes: crystalltone (A1) Sesquiterpenoids: dendronobilin B (A1); crystallinin (A2) Other kinds of compounds: monoaromatics; flavonoids; steroids; lignans; terpenes; nucleosides	Li et al. (2007), Wang et al. (2008, 2009b, 2011b)
D. cumulatum	Bibenzyls: tristin (A); cumulatin (A)	Majumder and Pal (1993)

Table 1 continued

Dendrobium species	Components	Refs.
D. densiflorum	Bibenzyls: moscatilin (A); gigantol (A); tristin (A); densiflorol A (B2) Phenanthrenes: moscatin (A1); denthyrsinin (A1); lhridinusiant (A2); densiflorol B (C1); cypripedin (C1) Fluorenones: dendroflorin (A1); dengibsin (A1) Coumarins: scoparone (B); scopoletin (B); scopolin (B); ayapin (C1); dihydroayapin (C2) Sesquiterpenoids: dendrodensiflorol (A1) Other kinds of compounds: flavonoids; monoaromatics	Fan et al. (2000, 2001), Zheng et al. (2000b), Tang et al. (2004), Dahmen et al. (1975)
D. draconis	Bibenzyls: gigantol (A); batatasin III (A) Phenanthrenes: 2,5,7-trihydroxy-4-methoxy-9,10-dihydrophenanthrene (A2); hircinol (A2); 5-methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone (C2)	Sritularak et al. (2011a)
D. falconeri	Bibenzyls: dendrofalconerol B (B3) Other kinds of compounds: monoaromatics	Sritularak and Likhitwitayawuid (2009)
D. farmerii	Fluorenones: dengibsin (A1) Coumarins: scoparone (B)	Majumder and Chakraborti (1989)
D. fimbriatum	Bibenzyls: moscatilin (A); chrysotobibenzyl (A); chrysotoxine (A); crepidatin (A) Phenanthrenes: confusarin (A1); fimbriatone (A1) Coumarins: scoparone (B); ayapin (C1) Sesquiterpenoids: denhydroshizukanolide (C3) Other kinds of compounds: monoaromatics; anthraquinones; steroids	Bi et al. (2001a, b, 2003), Majumder and Bandyopadhyay (2010), Qing et al. (2009), Talapatra et al. (1992)
D. findlayanum	Alkaloids: dendrobine (A1); 10-hydroxydendrobine (A1); nobiline (A4) Sesquiterpenoids: crystallinin (A2); findlayanin (A4)	Granelli et al. (1970), Qin et al. (2011)
D. friedricksianum	Alkaloids: dendramine (A1); *N*-isopentenyldendroxinium (A1); *N*-isopentenyl-6-hydroxydendroxinium (A1); nobiline (A4); 6-hydroxy-nobiline (A4)	Hedman et al. (1971)
D. fuscescens	Other kinds of compounds: monoaromatics	Talapatra et al. (1989)
D. gibsonii	Fluorenones: dengibsin (A1); dengibsinin (A1)	Talapatra et al. (1985)
D. gratiosissimum	Bibenzyls: moscatilin (A); gigantol (A); batatasin III (A); tristin (A); erianin (A); 3,4′,5-trihydroxybibenzyl (A); 3,4′-dihydroxy-5-methoxybibenzyl (A); 3,4-dihydroxy-4′,5-dimethoxy bibenzyl (A); isomoniliformine A (A); dengraols A (B5); dengraols B (B5) Other kinds of compounds: monoaromatics; steroids; flavonoids	Zhang et al. (2007a, 2008b), Wang et al. 2007)
D. hilderbrandii	Alkaloids: dendramine (A1); *N*-isopentenyldendroxinium (A1); *N*-isopentenyl-6-hydroxydendroxinium (A1); nobiline (A4); 6-hydroxy-nobiline (A4)	Elander and Leander (1971), Hedman et al. (1971)
D. huoshanense	Other kinds of compounds: flavonoids; monoaromatics	Chang et al. (2010)

Table 1 continued

Dendrobium species	Components	Refs.
D. loddigessi	Alkaloids: shihunidine (C); shihunine (D1)	Li et al. (1991), Ito et al. (2010)
	Bibenzyls: moscatilin (A); gigantol (A); batatasin III (A); loddigesiinol C (A); loddigesiinol D (A)	
	Phenanthrenes: moscatin (A1); 5-hydroxy-2,4-dimethoxyphenanthrene (A1); loddigesiinol A (A1); plicatol C (A2); lhridinusiant (A2); hircinol (A2); loddigesiinol B (B1)	
	Other kinds of compounds: steroids; lignans	
D. lohohense	Alkaloids: shihunine (D1)	Inubushi et al. (1964)
D. longicornu	Bibenzyls: moscatilin (A); gigantol (A); batatasin III (A); aloifol I (A); tristin (A); 3,3′,4-trihydroxybibenzyl (A); longicornuol B (A); 3,4′-dihydroxy-3′,4,5-trimethoxybibenzyl (A); 3,3′-Dihydroxy-4,5-dimethoxybibenzyl (A); cannabistilbene II (A); longicornuol A (B4); trigonopol A (B4)	Hu et al. (2008a, 2010), Chen et al. (2010), Hu (2007)
	Phenanthrenes: 2,5,7-trihydroxy-4-methoxy-9,10-dihydrophenanthrene (A2); coelonin (A2); hircinol (A2); dendronone (C2); ephemeranthoquinone (C2)	
	Other kinds of compounds: monoaromatics; steroids; flavonoids	
D. moniliforme	Alkaloids: monoline (A3)	Liu et al. (2007b), Zhao et al. (2003a, b), Bi et al. (2002, 2004), Zhao and Zhao (2003), Bae et al. (2004), Lin et al. (2000, 2001)
	Bibenzyls: moscatilin (A); 3,4′-dihydroxy-5-methoxybibenzyl (A); 3,4′-dihydroxy-3′,5-dimethoxybibenzyl (A); 3,4-dihydroxy-4′,5-dimethoxy bibenzyl (A); dendromoniliside E (A)	
	Phenanthrenes: 2,4,7-trihydroxy-9,10-dihydrophenanthrene (A2); 7-hydroxy-5,6-dimethoxy-1,4-phenanthrenequinone (C1); denbinobin (C1); moniliquinone (C3)	
	Sesquiterpenoids: dendromoniliside B (A1); dendromoniliside D (A1); dendroside F (A1); dendrobiumane B (A1); dendrobiumane D (A1); dendromoniliside C (A1); α-dihydropicrotoxinin (A1); picrotin (A1); dendrobiumane E (A1);dendrobiumane C (A2); dendromoniliside A (A6); dendrobiumane A (B); 10β,13,14-trihydroxyalloaromadendrane (B); dendroside A (B); dendroside C(B)	
	Other kinds of compounds: monoaromatics; lignans; steroids	
D. moscatum	Bibenzyls: moscatilin (A)	Majumder and Sen (1987a, b)
	Phenanthrenes: moscatin (A1)	

Table 1 continued

Dendrobium species	Components	Refs.
D. nobile	Alkaloids: dendrobine (A1); dendramine (A1); dendrine (A1); dendronobiline A (A1); 3-hydroxy-2-oxodendrobine (A1); dendroxine (A1); 6-hydroxy-dendroxine (A1); 8-hydroxy-dendroxine (A1); *N*-methyldendrobinium (A1); *N*-isopentenyldendrobinium (A1); dendrobine *N*-oxide (A1); *N*-isopentenyldendroxinium (A1); *N*-isopentenyl-6-hydroxydendroxinium (A1); nobiline (A4) Bibenzyls: moscatilin (A); gigantol (A); chrysotobibenzyl (A); chrysotoxine (A); crepidatin (A); batatasin III (A); tristin (A); 3-*O*-methylgigantol (A); dendrobin A (A); 4-hydroxy-3,3′,5-trimethoxybibenzyl (A); nobilin A (A); nobilin B (A); nobilin C (A); nobilin D (A); dendronophenol A (A); dendronophenol B (A); nobilin E (B3) Phenanthrenes: moscatin (A1); confusarin (A1); 2,5-dihydroxy-4,9-dimethoxyphenanthrene (A1); 2,6-dihydroxy-5,7-dimethoxyphenanthrene (A1); 2,5-dihydroxy-3,4,8-trimethoxyphenanthrene (A1); 2,8-dihydroxy-3,4,7-trimethoxyphenanthrene (A1); 2,3,5-trihydroxy-4,9-dimethoxyphenanthrene (A1); 3-hydroxy-2,4,7-trimethoxyphenanthrene (A1); 2,5-dihydroxy-3,4-dimethoxyphenanthrene (A1); flavanthrinin (A1); nudol (A1); bulbophyllanthrin (A1); fimbriol B (A1); plicatol A (A1); fimbriatone (A1); 4,5-dihydroxy-3,7-dimethoxy-9,10-dihydrophenanthrene (A2); 3-hydroxy-2,4,7-trimethoxy-9,10-dihydrophenanthrene (A2); 2,8-dihydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (A2); 2-hydroxy-4,7-dimethoxy-9,10-dihydrophenanthrene (A2); 2-hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (A2); 4,5-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (A2); lhridinusiant (A2); coelonin (A2); erianthridin (A2); ephemeranthol A (A2); ephemeranthol C (A2); hircinol (A2); flavanthridin (A2); 2,2′-dihydroxy-3,3′,4,4′,7,7′-hexamethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene (A2); denbinobin (C1) Fluorenones: dendroflorin (A1); denchrysan A (A1); dengibsin (A1); nobilone (A1) Sesquiterpenoids: dendronobilin B (A1); dendronobilin J (A1); dendronobilin L (A1); dendrodensiflorol (A1); dendroside F (A1); dendroside G (A1); dendronobilin C (A1); dendronobilin D (A1); dendronobilin E (A1); dendronobilin F (A1); dendronobilin M (A1); nobilomethylene (A1); 7,12-dihydroxy-5-hydroxymethyl-11-isopropyl-6-methyl-9-oxatricyclo[6.2.1.$0^{2,6}$]undecan-10-one-15-*O*-β-D-glucopyranoside (A1); dendronobiloside A (A5); dendronobiloside B (A5); 6α,10,12-trihydroxypicrotoxane (A5); 10,12-dihydroxypicrotoxane (A5); bullatantriol (A5); dendronobilin A (A6); dendronobilin K (A6); dendronobilin H(B); dendrobiumane A (B); 10β,13,14-trihydroxyalloaromadendrane (B); 10β,12,14-trihydroxyalloaromadendrane (B); dendroside A (B); dendroside B(B); dendroside D (B); dendronobilin G (C2); dendroside E (C4); dendronobiloside C (C5); dendronobiloside D (C5); dendronobilin I (C6); dendronobilin N (C6); dendronobiloside E (C6); dendrobane A (C6) Other kinds of compounds: monoaromatics; lignans; anthraquinones; terpenes; flavonoids	Inubushi et al. (1965), Suzuki and Keimatsu (1932), Wang et al. (1985), Yamamura and Hirata (1964), Okamoto et al. (1966a), Inubushi and Nakano (1965), Liu and Zhao (2003), Okamoto et al. (1966b, 1972), Hedman and Leander (1972), Hwang et al. (2010), Liu et al. (2007a), Miyazawa et al. (1997, 1999), Li et al. (2010c), Ye and Zhao (2002), Zhang et al. (2006c, d, 2007c, d, e, 2008c, d, e), Yang et al. (2007a), Lee et al. (1995), Yang and Xin (2006), Ye et al. (2002), Zhao et al. (2001), Shu et al. (2004a, b), Luo et al. (2006), Xu et al. (2008)

Table 1 continued

Dendrobium species	Components	Refs.
D. ochreatum	Other kinds of compounds: steroids	Behr et al. (1975), Behr and Leander 1976)
D. officinale	Bibenzyls: gigantol (A); 3-*O*-methylgigantol (A); 3,4′,5-trihydroxybibenzyl (A); 3,4′-dihydroxy-5-methoxybibenzyl (A); 4,4′-dihydroxy-3,5-dimethoxybibenzy (A); 3,4-dihydroxy-4′,5-dimethoxy bibenzyl (A); dendrophenol (A); dendromoniliside E (A); dendrocandin A (A); dendrocandin C (A); dendrocandin D (A); dendrocandin E (A); dendrocandin M (A); dendrocandin F (B3); dendrocandin G (B3); dendrocandin J (B3); dendrocandin K (B3); dendrocandin N (B6); dendrocandin O (B6); dendrocandin P (B6); dendrocandin Q (B6); dendrocandin B (B6); dendrocandin I (B6); dendrocandin R (B7)	Guan et al. (2009), Li et al. (2008, 2009a, d, e, 2010a, b), Li (2009), Sritularak and Likhitwitayawuid (2009), Wang et al. (2010a)
	Phenanthrenes: confusarin (A1); 2,5-dihydroxy-3,4-dimethoxyphenanthrene (A1); 2,3,4,7-tetramethoxyphenanthrene (A1); nudol (A1); bulbophyllanthrin (A1); 2,4,7-trihydroxy-9,10-dihydrophenanthrene (A2); denbinobin (C1); dendrocandin H (C4); dendrocandin L (C4)	
	Sesquiterpenoids: aduncin (A1)	
	Other kinds of compounds: monoaromatics; lignans; flavonoids; terpenes; steroids	
D. parishii	Alkaloids: dendroparine (E);	Leander and Lüning (1968)
D. pierardii	Alkaloids: pierardine (D2);	Elander et al. (1971)
D. plicatile	Bibenzyls: batatasin III (A); 3-*O*-methylgigantol (A)	Yamaki and Honda (1996), Honda and Yamaki (2000, 2001)
	Phenanthrenes: moscatin (A1); 2,6-dihydroxy-5,7-dimethoxyphenanthrene (A1); 3-hydroxy-2,4,7-trimethoxyphenanthrene (A1); plicatol A (A1); plicatol C (A2); 2-hydroxy-5,6,7-trimethoxy-9,10-dihydrophenanthrene (A2); lhridinusiant (A2); erianthridin (A2); hircinol (A2); 4,4′,7,7′-tetrahydroxy-2,2′-dimethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene (A2); ephemeranthoquinone (C2)	
D. polyanthum	Bibenzyls: moscatilin (A); gigantol (A); batatasin III (A)	Hu et al. (2009)
	Phenanthrenes: moscatin (A1); 2,4,7-trihydroxy-9,10-dihydrophenanthrene (A2); hircinol (A2)	
	Sesquiterpenoids: corchoionoside C (D1)	
	Other kinds of compounds: steroids	
D. primulimun	Alkaloids: dendroprimine (B2); hygrine (C)	Blomqvist et al. (1972), Luning and Leander (1965), Hu (2007)
	Sesquiterpenoids: corchoionoside C (C1)	
	Other kinds of compounds: steroids	
D. rotundatum	Bibenzyls: batatasin III (A)	Majumder and Pal (1992)
	Phenanthrenes: moscatin (A1); 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene (A1); nudol (A1); plicatol C (A2); 2,7-Dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene (A2)	

Table 1 continued

Dendrobium species	Components	Refs.
D. secundum	Bibenzyls: moscatilin (A); 4,5,4′-Trihydroxy-3,3′-dimethoxybibenzyl (A); brittonin A (A)	Sritularak et al. (2011b)
D. snowflake	Alkaloids: mubironine A (A1); mubironine B (A1); mubironine C (A2)	Morita et al. (2000)
	Sesquiterpenoids: flakinin B (A2); flakinin A (A3);	
D. sonia	Bibenzyls: gigantol (A); 3-*O*-methylgigantol (A)	Huang et al. (2000)
	Phenanthrenes: confusarin (A1); nudol (A1); lhridinusiant (A2)	
D. thyrsiflorum	Bibenzyls: moscatilin (A); gigantol (A); tristin (A)	Zhang et al. (2004, 2005a, b), Liu et al. (2011), Wrigley (1960)
	Phenanthrenes: moscatin (A1); denthyrsinin (A1); denthyrsinol (A1); hircinol (A2); densiflorol B (C1); denthyrsinone (C1)	
	Fluorenones: dendroflorin (A1); denchrysan A (A1); dengibsin (A1); denchrysan B (A2)	
	Coumarins: scoparone (B); scopoletin (B); scopolin (B); xeroboside (B); denthyrsin (B); ayapin (C1)	
	Other kinds of compounds: monoaromatics; flavonoids; steroids; terpenes; anthraquinones	
D. trigonopus	Bibenzyls: moscatilin (A); gigantol (A); tristin (A); trigonopol B (B3); trigonopol A (B4)	Hu (2007), Hu et al. (2008b), Zhang et al. (2005c)
	Phenanthrenes: moscatin (A1); hircinol (A2)	
	Fluorenones: dendroflorin (A1)	
	Other kinds of compounds: lignans; steroids; flavonoids	
D. wardianum	Alkaloids: dendrowardine (A5)	Glomqvist et al. (1973)

[a] Code for chemical skeleton of each kind of compounds

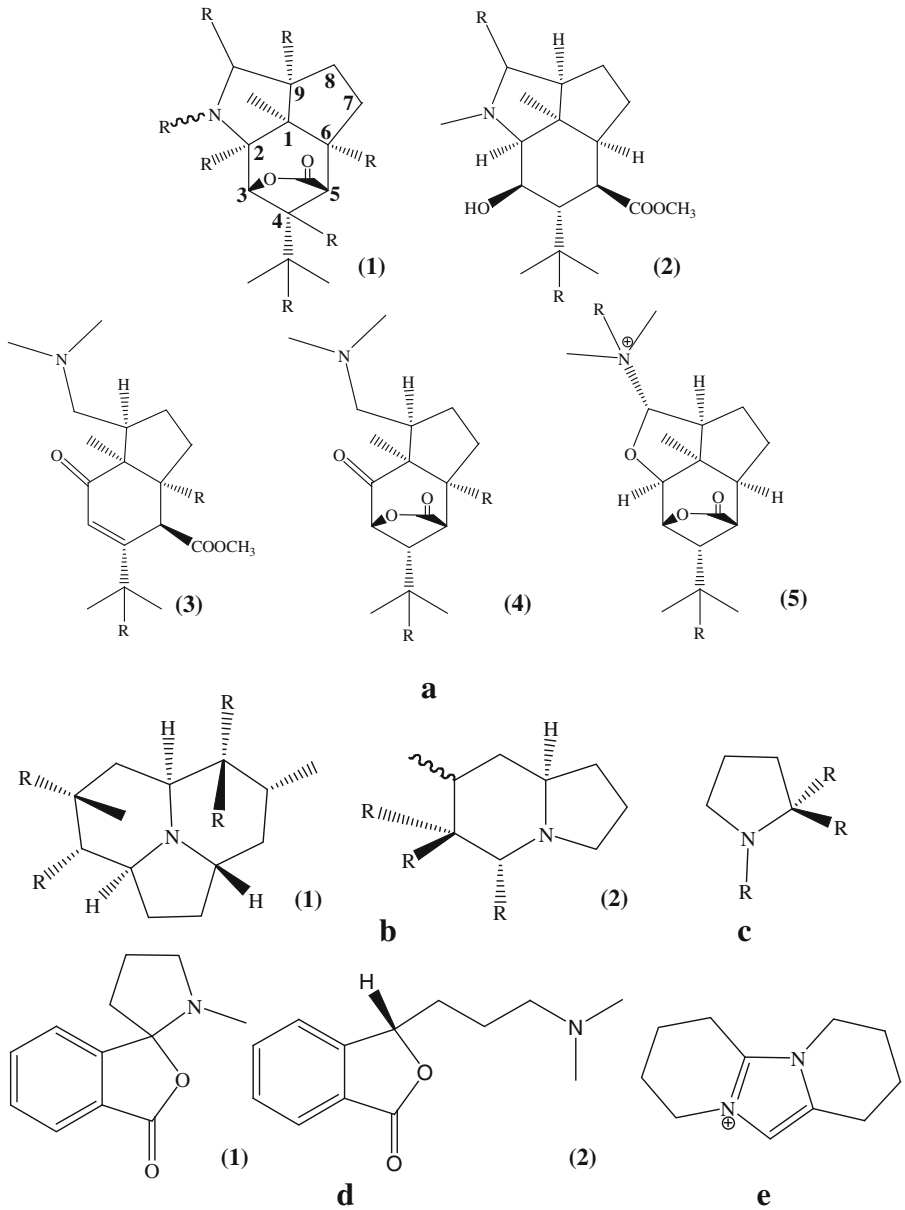


Fig. 2 Chemical skeletons of alkaloids in *Dendrobium*. **a** Sesquiterpenoid type; **b** indolizidine type; **c** pyrrolidine type; **d** phthalide type; **e** imidazole type

five-membered C2–C9-linked N-heterocycle and a C3–C5-linked lactonic ring (Fig. 2a, 1). Various substituent groups, frequently a methyl group, appear on the N atom. In particular, the C2–C9 N-heterocycle and C3–C5 lactonic ring are open in some sesquiterpenoid alkaloids from *Dendrobium* species (Fig. 2a, 2–4). Indolizidine alkaloids in *Dendrobium* are formed by mixed-joint multiple substituted piperidine(s) and pyrrolidine with mutual C and N atoms (Fig. 2b, 1–2). Substituent groups, such as methyl, hydroxyl, acetyl and phenyl, are always present on the piperidine(s). Indolizidine alkaloids have only been found in *Dendrobium crepidatum* and *D. primulinum*. The structures of pyrrolidine alkaloids, isolated mainly from *D. chrysanthum*, are simple. Only one or two pyrrolidines linked by di-substituted acetonyl, with some ordinary substituents, such as cinnamoyl, methyl and acetonyl, compose this kind of alkaloid

(Fig. 2c). The other two types of alkaloids, phthalides and imidazoles, are rarely found in the four *Dendrobium* species investigated (Table 1; Fig. 2d, e).

Main aromatics

A large number of aromatic compounds, represented by bibenzyls, phenanthrenes, fluorenones and coumarins, have been reported from *Dendrobium*.

Bibenzyls are widespread in different *Dendrobium* species. For example, moscatilin and gigantol have been isolated from nearly twenty species of *Dendrobium* (Table 1). The structures of the major bibenzyls in *Dendrobium* are simple and generally consist of a basic framework, bibenzyl, also known as 1,2-diphenylethane, with substituents, always located at the para- and/or meta-positions on the benzene ring C atoms which are substituted by ethyl (Fig. 3a). The

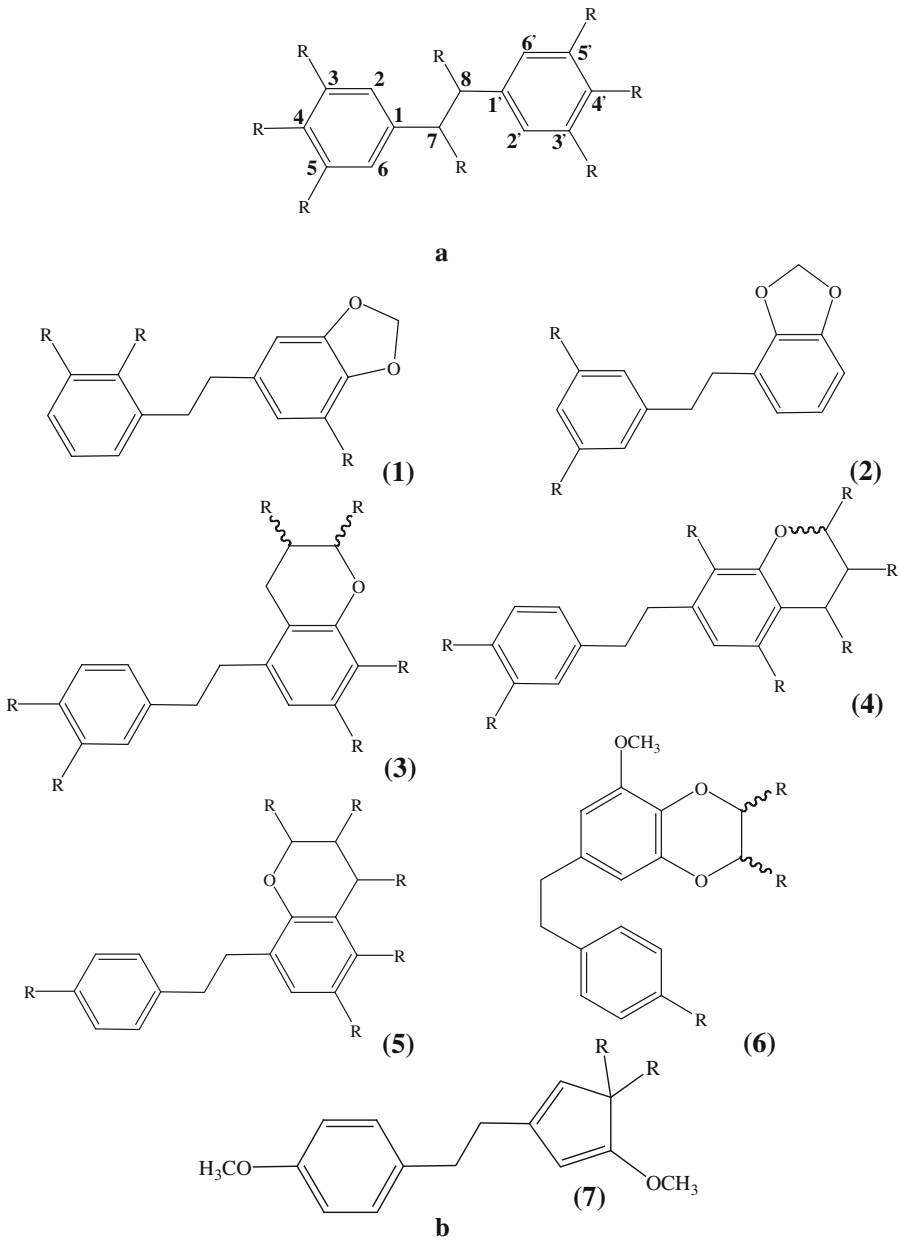


Fig. 3 Chemical skeletons of bibenzyls in *Dendrobium*. **a** Simple bibenzyls; **b** intricate bibenzyls

substituents are frequently hydroxyl and/or methoxyl, and sometimes phenyl, phenoxyl, phenacetyl and glucosyl. The total number of these groups is between 3 and 6. The structural diversity of bibenzyls in *Dendrobium* depends on the type, number and position of these substituents. In particular, the two C atoms in ethyl (C7 and C8) can rarely be substituted. Interestingly, none of the bibenzyls in *Dendrobium* has been found with mono-substitution. The same situation also occurs with phenanthrenes (except for phenanthraquinones) and fluorenones in *Dendrobium*. The other bibenzyls found in *Dendrobium* species possess more intricate structural characteristics (Fig. 3b). In these bibenzyl derivatives, one of the two benzene rings in bibenzyl is always combined with an intricately substituted oxygenic (benzo-) heterocycle. This kind of bibenzyl has been found in abundance in *D. officinale*.

Most natural phenanthrenes in *Dendrobium* species appear to be hydroxyl- and/or methoxyl-substituted

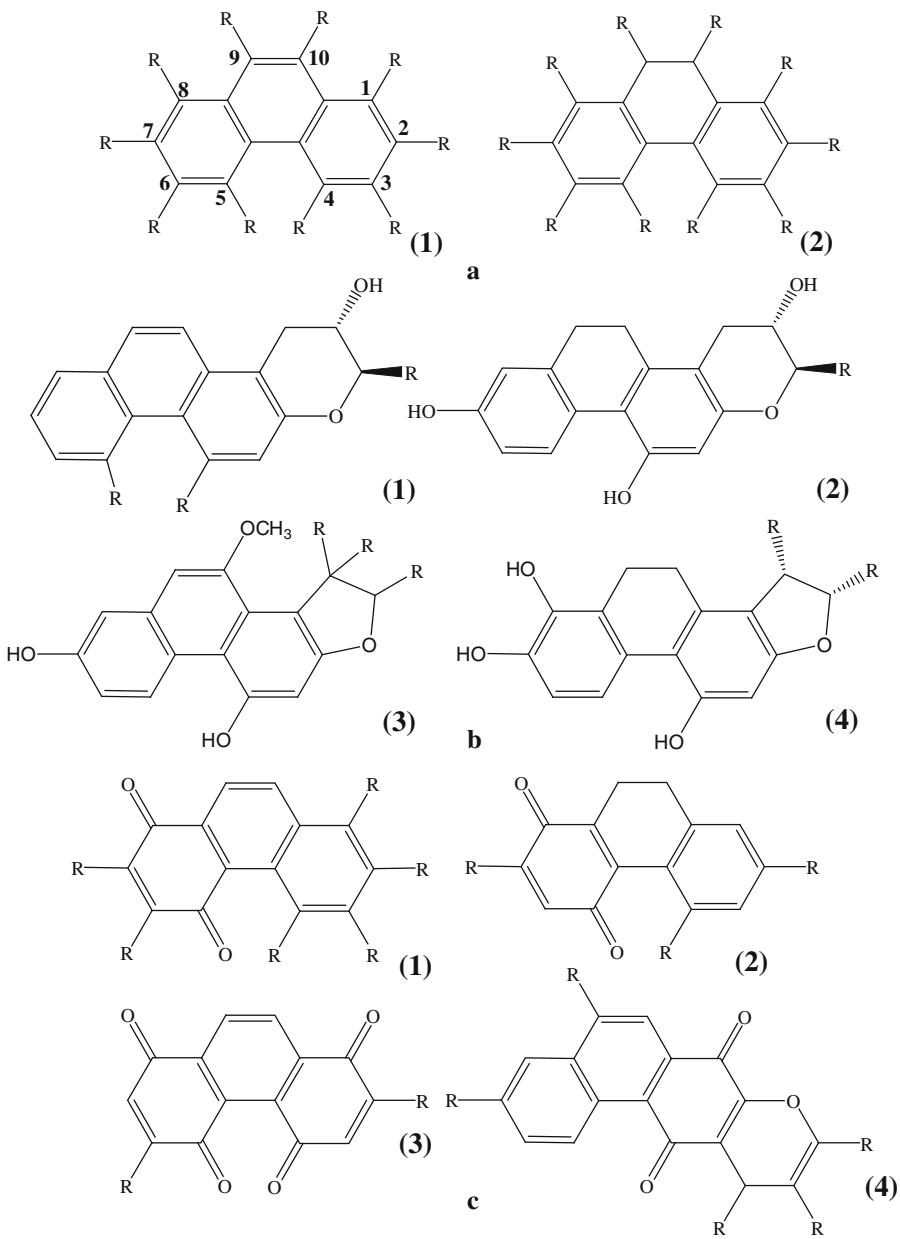


Fig. 4 Chemical skeletons of phenanthrenes in *Dendrobium*. **a** Simple phenanthrenes; **b** intricate phenanthrenes; **c** phenanthrenequinones

9,10-dihydro or dehydro derivatives (Table 1; Fig. 4a). The numbers of hydroxyl and methoxyl moieties are also between 3 and 6, and can be found usually at C2–7 and sometimes at C1, C8 and C9. Furthermore, C4 and C5 in some phenanthrenes in *Dendrobium* can be linked with a di-substituted ester or methoxyl group to form an oxygen-bearing hexatomic ring or lactonic ring. Additionally, some novel types of 9,10-dehydro and dihydro-phenanthrene combined with miscellaneous substituted pyran or furan have also been isolated and identified from several *Dendrobium* species (Fig. 4b). Another type of phenanthrenes found in *Dendrobium* is the group of phenanthraquinones. 1,4-phenanthraquinone and 9,10-dihydro-1,4-phenanthraquinone are frequent skeletons of phenanthraquinones in *Dendrobium* (Table 1; Fig. 4c).

Fluorenones and coumarins are widespread in *Dendrobium* species as well. However, the quantities of these two categories of aromatics in *Dendrobium* are much lower than bibenzyls and phenanthrenes. Universally, C1–8 of the fluorenones in *Dendrobium* are selectively substituted by 3 to 5 hydroxyls and methoxyls (Fig. 5a). As to coumarins in *Dendrobium* species, they generally consist of a coumarin skeleton with C5 and C6, sometimes C2, substituents, mostly hydroxyls and methoxyls (Fig. 5b, c). It is worth mentioning that *D. densiflorum* and *D. thyrsiflorum* are the richest resources for coumarins.

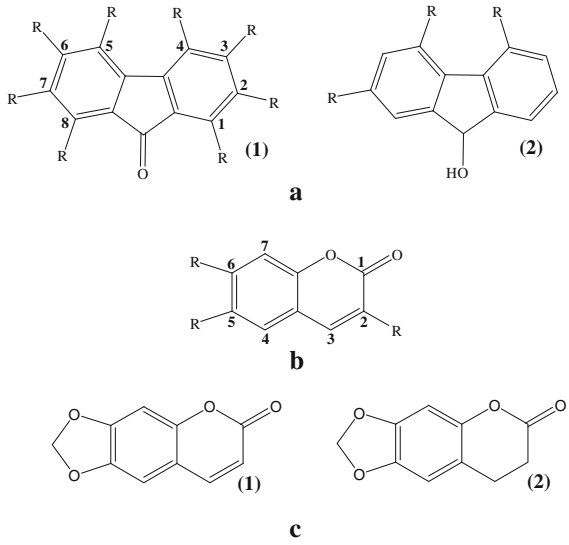


Fig. 5 Chemical skeletons of fluorenones (**a**) and coumarins (**b, c**) in *Dendrobium*

Sesquiterpenoids

Sesquiterpenoids are also frequently found in *Dendrobium* species. Unlike aromatics in *Dendrobium*, which are widely distributed, sesquiterpenoids have been found in only nine *Dendrobium* species so far, and the majority is only found in *D. nobile* and *D. moniliforme* (Table 1). As shown in Table 1, the picrotoxane type is the most common sesquiterpenoid isolated from *Dendrobium* (Fig. 6a). Similar to sesquiterpenoid alkaloids in *Dendrobium*, the picrotoxane skeleton is sometimes combined with C2–C9- and/or C3–C5-linked, rarely C5–C7 or C5–C10-linked, lactonic rings (Fig. 6a). General substituents, mostly hydroxyl and/or hydroxymethyl, and occasionally methyl, methoxyl, carboxyl, carbonyl or glycosyl, are found on the picrotoxane-type skeleton. In particular, C5 and C9 in the picrotoxane skeleton can be linked with a carbonyl (Fig. 6a). The alloaromadendrane type, another kind of sesquiterpenoid (Fig. 6b), is also rich in *D. nobile* and *D. moniliforme*. Methyl is always attached to the C4 of the alloaromadendrane skeleton while several kinds of substitutions frequently happen at C10 and C11. Furthermore, other types of sesquiterpenoids, such as cyclocopacamphane, cadinene, emmotin and muurolene, have also been found, mainly in *D. nobile* (Fig. 6c).

Other small molecules

In addition to the above mentioned characteristic components in *Dendrobium*, other common types of micro-molecular compounds have also been isolated from many *Dendrobium* species (Table 1) and most of them are mono-aromatics, lignans, steroids, flavonoids, or anthraquinones.

Polysaccharides

Polysaccharides, a class of carbohydrate consisting of numerous (usually more than ten) monosaccharides linked by glycosidic bonds in branched or unbranched chains, are usually considered as one of the most important active compounds in medicinal plants. Polysaccharides always present with significant amounts in *Dendrobium*, even representing up to nearly 50% of the total dry weight in some species, e.g. *D officinale* (Li and Xu 1990), and have been

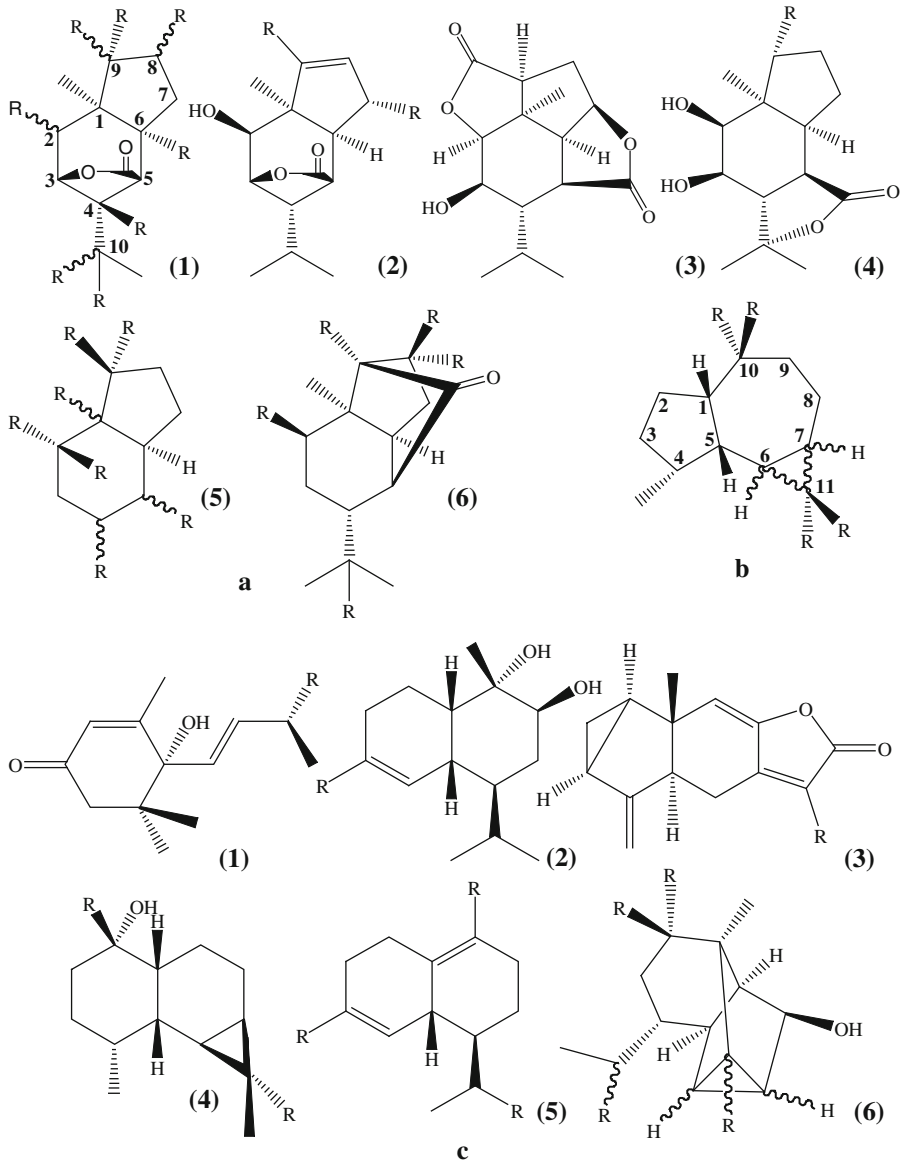
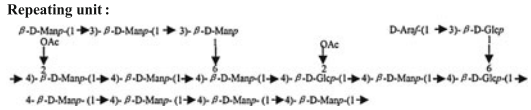
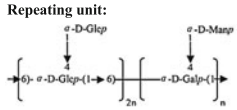
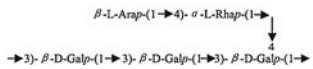


Fig. 6 Chemical skeletons of sesquiterpenoids in *Dendrobium*. **a** Picrotoxane type; **b** alloaromadendrane type; **c** other types

experimentally proven to exert multiple biological properties, such as immuno-modulation, anti-tumor and anti-oxidant activity (Ng et al. 2012).

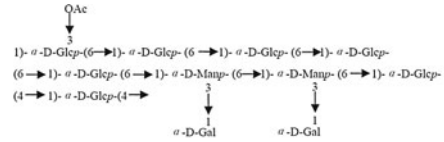
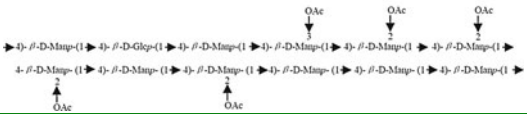
To date, several kinds of polysaccharides have been isolated and purified from *Dendrobium* species (Table 2). However, compared with studies on micro-molecular compounds, reports on the chemistry with regard to isolation, purification, and in-depth structural elucidation of the polysaccharides in *Dendrobium* are limited and only focus on six frequently used species in China, i.e., *D. officinale*, *D. nobile*, *D. huoshanense*, *D. aurantiacum* var. *denneanum*, *D. moniliforme* and *D. aphyllum*, as summarized in Table 2. As shown in Table 2, there are discrepancies in reports of the molecular weights of the purified polysaccharides from these *Dendrobium* species. Glucose, mannose and galactose, which are most frequently found, are the three main monosaccharides comprising the polysaccharides isolated from *Dendrobium*. In addition, other monosaccharides and uronic

Table 2 Polysaccharides isolated and purified from *Dendrobium* spp.

Occurence	Name	MW (Da)	Molar Ratio	Stuctural characteristics (backbone, branch and repeating units)	Ref.
Dendrobium officinale	CI	1.0×10^6	---[a]	**Backbone :** Several → 3)-β-D-Manp-(1 → and one → 4)-β-D-Glcp-(1 → **Branch:** → 4)-β-D-Glcp-(1 →	(Wang et al. 1988)
	CII	5.0×10^5	---		
	CIII	1.2×10^5	---		
	DT2	7.4×10^5	Glc :Gal:Xyl:Ara:Man= 5.9:1.0:1.0:0.8:0.5	**Backbone:** → 4)-α-D-Glcp-(1 →	(Yang et al. 2004c)
	DT3	5.4×10^5	Glc:Gal:Xyl:Ara:Man=7.9:1.3:1. 0:0.5:0.7		
	DOP-1-A1	1.3×10^5	Man:Glc:Ara=40.2:8.4:1.0	**Repeating unit :** 	(Hua et al. 2004)
	DCPP1a-1	1.89×10^5	Man:Glc=7.015:1.000	---	(He et al. 2007)
	DOPW-1	7.8×10^4	Rha:Ara:Xyl:Man:Gal:Glu=0.09: 1.00:0.14:0.18:2.73:2.16	---	(Chen et al. 2011)
	DOPW-2	3.7×10^4	Rha:Ara:Xyl:Man:Gal:Glu=0.06: 1.00:0.22:0.46:4.43:1.02	---	
	DOPS1-1	2.87×10^5	Rha:Ara:Xyl:Man:Gal:Glu=0.03: 1.00:0.12:0.04:2.05:0.29	---	
	DOPS1-2	3.51×10^5	Rha:Ara:Xyl:Gal:Glu=0.44:1.00: 0.21:3.16:0.26	---	
	DOPS1-3	3.35×10^5	Rha:Ara:Xyl:Man:Gal:Glu=0.30: 1.00:0.32:0.15:2.37:0.37	---	
	DOPS1-4	1.71×10^5	Rha:Ara:Xyl:Gal:Glu=0.87:1.00: 0.20:2.20:0.24	---	
	DOP-1	5.34×10^5	Man:Glc:Gal:Ara=3.13:1.34:0.02 :0.01	---	(Xia et al. 2012)
	DOP-2	1.60×10^5	Man:Glc:Gal:Ara=3.13:1.24:0.12 :0.02	---	
D. nobile	DNP	8.76×10^4	Rha:Ara:Xyl:Man:Glc:Gal=1.0:2 .8:2.2:30.8:117.9:31.8	**Repeating unit:** 	(Luo et al. 2009)
	DNP-W2	1.8×10^4	Glc:Man:Gla=6.1:2.9:2.0	**Backbone:** → 4)-β-D-Glcp-(1 → and → 4)-β-D-Manp-(1 → and → 6)-β-D-Glcp-(1 → **Branch:** α-D-galactopyranoyl	(Wang et al. 2010d)
	DNP-W3	7.1×10^5	Gal:Rha:Ara=3.1:1.1:1.0	**Repeating unit:** 	(Wang et al. 2010b)
	DNP1-1	1.36×10^5	Rha:Ara:Xyl:Man:Glc:Gal=2.11: 3.54:0.89:12.97:44.65:35.85	---	(Luo et al. 2010)
	DNP2-1	2.77×10^4	Man:Glc:Gal=16.99:53.26:29.74	---	
	DNP3-1	1.18×10^4	Rha:Ara:Man:Glc:Gal=3.76:8.48 :6.55:12.58:68.63	---	
	DNP4-2	1.14×10^4	Rha:Ara:Man:Glc:Gal=12.59:4.2 0:11.64:23.47:48.10	---	
	DNP-W5	4.6×10^5	Man:Glc:Gal:Xyl:Rha:GalA=3.1: 8.1:8.2:0.6:4.2:3.9	**Backbone:** → 4)-α-GalAp-(1 → 2)-α-Rhap-(1 → ; **Branches:** galactosyl, mannosyl, glucosyl, and xylosyl	(Wang et al. 2010c)

acids, such as arabinose, rhamonose, xylose and galaturonic acid, also occasionally appeared in the backbone or branched and terminal residues of purified *Dendrobium* polysaccharides. Moreover, glucosidic bonds in *Dendrobium* polysaccharides are complicated, mainly including 1 → 4, 1 → 6 or

Table 2 continued

D. huoshanense	HPS-1B23	2.2×10^4	Glc:Man:Gal=31:10:8	**Repeating unit:** 	(Zha et al. 2007)
	---	9.7×10^3	Man:Glc=10:1	**Repeating unit:** 	(Hsieh et al. 2008)
D. aurantiacum var. *denneanum*	DDP1-1	5.15×10^4	Ara:Xyl:Man:Glc:Gal=1.00:2.82:57.11:140.82:7.76	---	(Fan et al. 2009)
	DDP2-1	2.61×10^4	Ara:Xyl:Man:Glc:Gal=1.00:1.62:1.18:77.5:7.79	---	
	DDP3-1	6.95×10^3	Ara:Man:Glc:Gal=1.00:1.03:8.84:2.00	---	
D. moniliforme	DMP1a-1	2.8×10^4	Glc:Man=1:4.798	---	(Chen et al. 2003)
	DMP2a-1	6.0×10^3	Glc:Man=5.1:0.5	**Backbone:** 	(Xu et al. 2004)
	DMP4a-1	3.05×10^3	Glc:Man:Rha:Ara:Gal=2.873:2.850:1.762:1.279:1	---	(Chen et al. 2005)
D. aphyllum	AP-1	8.63×10^4	---	---	(Zhao et al. 1994)
	AP-2	6.15×10^4	---	---	
	AP-3	4.31×10^4	---	---	

Man*p*, mannopyranosyl; Glc*p*, glucopyranosyl; OAc, O-acetyl; Ara*f*, arabinofuranosyl; Gal*p*, galactopyranosyl; Ara*p*, arabinopyranosyl; Rha*p* rhamnopyranosyl

Man, mannose; Glc, glucose; Ara, arabinose; Gal, galactose; Xyl, xylose; Rha, rhamnose; GalA, galacturonic acid

[a] No informations

1 → 3 linkages. Pyranosyls with α- or *β*-configuration are prevalent in the polysaccharides found in *Dendrobium*.

Bioactivity

According to traditional Chinese medical theory, *Dendrobium* is an herbal tonic for supplementing the stomach, promoting the production of body fluids, nourishing *Yin*, and clearing heat. At present, accumulating studies provide evidence that *Dendrobium* demonstrates extraordinarily comprehensive bioactivities, involving the immune, nervous, cardiovascular, endocrine, gastrointestinal and urinary systems (Ng et al. 2012).

Nonetheless, bioactivity studies on *Dendrobium* still suffer from serious problems. All too often, pharmacological effects of *Dendrobium* extracts or its pure components have been tested and verified only by in vitro experiments. Crucial factors that might directly influence in vivo efficacy, such as bioavailability and pharmacokinetics, can not be considered by in vitro experiments. Thus, to assess the actual activity of *Dendrobium*, in vivo experiments must be executed. To provide an example, gigantol, a bibenzyl that normally occurs in *Dendrobium*, has been reported to possess multiple positive effects that correlate with molecular mechanisms according to in vitro experiments, including anti-cataract, anti-tumoral, anti-mutagenic, anti-inflammatory, and antioxidant effects (Wei et al. 2011; Won et al. 2006; Miyazawa et al. 1997; Simmler et al. 2009). However, few in vivo studies have been carried out to further confirm the bioactivity of gigantol.

Secondly, the concentration-effect paradigm, also called the dose–response relationship, of *Dendrobium* extracts or components should be a factor of concern in evaluating studies. For example, many reports state that purified polysaccharides from different *Dendrobium* plants present competitive antioxidant activity (Lin et al. 2003). However, the dosages are often

amazingly unreasonable. DFHP, a water-soluble polysaccharide isolated from *D. fimbriatum*, was tested for its in vitro antioxidant activity (Luo and Fan 2011). In the scavenging activity on ABTS assay, DFHP showed a high scavenging effect on ABTS at 3.0 mg/mL, reaching 90.05%, which was close to that of vitamin C in the same concentration ($P < 0.05$). However, it should be noticed that the scavenging effect on ABTS of vitamin C at 0.25 mg/mL (about 90%) was already close to the level achieved at 3.0 mg/mL, but obviously much stronger than that of DFHP at 0.25 mg/mL (less than 30%). In other words, the scavenging effect on ABTS of DFHP at 3.0 mg/mL was similar with that of vitamin C at just 0.25 mg/mL. Generally, extraordinarily high concentrations of an extract or a natural product can get a "satisfactory" pharmacological response (Gertsch 2009). Unfortunately, the conclusion "the results indicated that DFHP had strong scavenging power for ABTS radicals and should be explored as novel potential antioxidants" was readily obtained when the similar scavenging effects on ABTS of DFHP and vitamin C were compared at a considerably high concentration (3.0 mg/mL). Additionally, beyond the concentration-effect paradigm, the yield of extracts or refined single components in *Dendrobium* herbal materials needs to be determined and calculated in order to determine potentially effective dosages for whole herbal material or to evaluate the feasibility of using herbal materials as sustainable resources. This issue has been ignored in most related published papers.

Thirdly, apart from pure components, most in vitro and in vivo pharmacological studies on *Dendrobium* used crude extracts, such as aqueous extracts, alcohol extracts, crude polysaccharides and total alkaloids (Lin et al. 2003). However, the specific bioactive substances in these crude extracts are obscure, and such information is not helpful for development of novel natural products from *Dendrobium* medicinal plants because it is difficult to standardize the crude extracts. Thus, crude *Dendrobium* extracts should be further investigated for particular components that are responsible for bioactivity. Furthermore, the interaction between components in crude *Dendrobium* extracts should also be studied to reveal the scientific basis that multiple components interact to create holistic therapeutic actions in traditional Chinese medicines.

Quality control

Qualitative analysis

Authentication

Due to the distinct chemical components, bioactivities and clinical effects of different *Dendrobium* species, authentication of *Dendrobium* is crucial and the first step for implementing its rational administration as a medicine. Traditional morphological and microscopic approaches along with molecular techniques used for *Dendrobium* authentication have been reviewed in detail (Zhang et al. 2005d). On the other hand, the diverse chemical components of different *Dendrobium* herbs make it possible to identify and discriminate *Dendrobium* species by chemical methods. Recently, multiple chromatographic fingerprints, such as high performance liquid chromatography (HPLC) (Zhang et al. 2003b), capillary electrophoresis (CE) (Zha et al. 2009), gas chromatography (GC) (Wang et al. 2011a) and thin-layer chromatography (TLC) (Wang et al. 2003), have been readily exploited for successful identification and discrimination of five *Dendrobium* species. In addition, some spectroscopic methods such as ^{1}H nuclear magnetic resonance (^{1}H-NMR) (Zhang et al. 2007b), infra-red spectrum (IR) (Li et al. 2009c), near infra-red spectrum (NIR) (Wang et al. 2009a) and ultraviolet spectrum (UV) (Teng et al. 2009) are also being employed for fingerprint analysis and/or discrimination of *Dendrobium* species.

In general, though extensive work has been conducted for authentication of *Dendrobium* species, problems still exist. Morphological and microscopic identification is very limited for *Dendrobium* herbs or processed products with similar macroscopic and anatomical characteristics. Molecular methods are quite effective, but might be not appropriate for routine use owing to the high cost. In spite of the feasibility of using chromatography for routine sample authentication, current and future studies on the development of fingerprint methods for authentication of *Dendrobium* species should be more comprehensive and be concerned with not only species, but also other factors that could cause chemical inconsistency, such as sample localities, harvesting time, and processing methods. Spectroscopic methods could be used for ordinary discrimination of *Dendrobium*

species, but they are very limited in *Dendrobium* authentication due to their poor specificity. Hence, abundant exploratory studies are still needed.

Qualitative analysis of polysaccharides

Quality control of polysaccharides remains a challenge because of their complicated structures and macro-molecular mass. Generally, isolation and purification followed by complete structural characterization is the most reliable method for quality evaluation of polysaccharides in medicinal herbs (Table 2). However, this procedure is complex and time consuming. Therefore, over the past 20 years, rapid and convenient methods for qualitative analysis of polysaccharides in *Dendrobium* have been developed, in which structural information of the investigated polysaccharides could be partially represented in different ways. Pre-column derivatization HPLC (Zhou and Lv 2011), TLC (Huang and Ruan 1997), GC (Luo et al. 2011) and derivatization polyacrylamide gel electrophoresis (PAGE) (Zha et al. 2012) analysis based on the constituent saccharides profiles produced by total or partial acid hydrolysis have been frequently used for characterization and quality control of crude polysaccharides from different *Dendrobium* herbs. However, the selectivity of acid hydrolysis for different glycosidic bonds is poor, which limits the structural characterization for various polysaccharides. Consequently, several novel methods were established, in which polysaccharides from *Dendrobium* were selectively hydrolyzed by specific carbohydrases, especially glycosidases, with more moderate conditions. A new "saccharide mapping" based on enzymatic (carbohydrase) digestion and subsequent chromatographic analysis of enzymatic hydrolysate was successfully employed for discrimination of crude polysaccharides from different *Dendrobium* species as well as the same species grown in different localities (Xu et al. 2011). Analogously, enzymatic fingerprints derived from carbohydrase hydrolysis followed by PAGE analysis were also created for species and locality identification of *Dendrobium* (Zha et al. 2012). These methods, based on specific glycosidic bonds, provide a different approach to the concise discrimination of polysaccharides from various origins and are helpful for assessing the pharmaceutical or therapeutic quality of polysaccharides in *Dendrobium*.

Quantitative analysis

Polysaccharides, alkaloids and aromatics have been proven to be largely responsible for the many biological activities of *Dendrobium* (Ng et al. 2012). Thus, quantitative analysis for the quality control of *Dendrobium* has mostly focused on these kinds of compounds. To date, a series of analytical methods have been employed and reported to quantify the contents of active components in various *Dendrobium* species. However, it should be noted that although sesquiterpenoids are also widely distributed in *Dendrobium* with proved bioactivities, studies on the quantitative analysis of sesquiterpenoids in *Dendrobium* has not been carried out yet.

Colorimetry and titration

The contents of total alkaloids and polysaccharides in *Dendrobium* have been determined by colorimetry and potentiometric titration (Li et al. 2002; Sun et al. 2009; Zhang et al. 2001; Zhu et al. 2010a, b). However, these methods, though simple and rapid, are sometimes unreliable due to the effects of uncontrolled experimental conditions (Xu 2001; Hua et al. 2010).

Chromatography

Currently, HPLC coupled with different detectors, such us UV and MS, has become the preferred analytical technique for separation and quantification of markers from complicated Chinese medicinal material extracts, due to its many advantageous features, including high resolution, favourable reproducibility and powerful maneuverability (Liang et al. 2009). HPLC methods for quantitative analysis of *Dendrobium* are summarized in Table 3. It can be seen that aromatic compounds, e.g., bibenzyls, phenanthrenes, fluorenones and coumarins, and alkaloids are always selected as chemical markers in HPLC quantitative analysis for the quality control of *Dendrobium* species. UV detection is mostly employed in these methods. Electrospray ionization (ESI)–mass spectrometry (MS) detection is seldomly used in HPLC analysis for further structural elucidation of targeted compounds in the quality control of *Dendrobium*. However, differing from aromatics with intense absorption in the ultraviolet region, alkaloids of

Table 3 HPLC applications on quantitative analysis for quality control of *Dendrobium* spp.

Analytes	Samples	Extraction method	Column	Mobile phase	Analytical time (min)	Detection	Refs.
Bibenzyls, fluorenones and phenanthrenes (11 chemical markers)	31 *Dendrobium* species	Ultrasonic extraction with 80% (*v:v*) methonal aqueous solution	ODS (Beckman Coulter™) (4.6 mm × 250 mm, 5 μm)	Acetonitrile–1 ‰ TFA, gradient elution Flow rate: 1.0 mL/min	45	UV 280 nm	Yang et al. (2006c)
Bibenzyls, phenanthrenes and coumarins (9 chemical markers)	*D. aurantiacum* var. *denneanum*	Ultrasonic extraction with 80% (*v:v*) methonal aqueous solution	ODS (Beckman Coulter™) (4.6 mm × 250 mm, 5 μm)	Acetonitrile–5 ‰ formic acid, gradient elution Flow rate: 1.0 mL/min	50	UV 280 nm ESI–MS	Yang et al. (2007e)
Bibenzyls, fluorenones and phenanthrenes (6 chemical markers)	*D. thyrsiflorum*	Soak and then ultrasonic extraction with 80% (*v:v*) methonal aqueous solution	ODS (Beckman Coulter™) (4.6 mm × 250 mm, 5 μm)	Acetonitrile–1 ‰TFA, gradient elution Flow rate: 1.0 mL/min	55	UV 280 nm	Yang et al. (2008)
Bibenzyls, fluorenones and phenanthrenes (6 chemical markers)	*D. chrysotoxum*	Refluxing extraction with chloroform	ODS (Shim-Pack CLC) (4.6 mm × 200 mm)	Methanol–water (58:42) Flow rate: 1.0 mL/min	35	UV 237 nm	Yang et al. (2005a)
Bibenzyls and phenanthrenes (3 chemical markers)	16 *Dendrobium* species	Refluxing extraction with chloroform	ODS (DICP, China) (4.6 mm × 240 mm)	Methanol–acetonitrile–water (60:60:165) Flow rate: 1.2 mL/min	40	UV 237 nm	Ma et al. (1994a)
Bibenzyls (2 chemical markers)	15 *Dendrobium* species	Refluxing extraction with chloroform	ODS (Shim-Pack CLC) (4.6 mm × 200 mm, 5 μm)	Methanol–water (60:40) Flow rate: 1.0 mL/min	40	UV 237 nm	Ding et al. (2008)
Bibenzyls (2 chemical markers)	18 *Dendrobium* species	Ultrasonic extraction with methonal	RP-18 (Waters XTerra™) (4.6 mm × 250 mm, 5 μm)	Acetonitrile–0.1 ‰ TFA, gradient elution Flow rate: 1.0 mL/min	35	UV 230 nm	Xu et al. (2010a)
Bibenzyls (2 chemical markers)	6 *Dendrobium* species	Ultrasonic extraction with 60% (*v:v*) methonal aqueous solution	C18 (Shimadzu) (2.1 mm × 150 mm, 5 μm)	Acetonitrile with 1 ‰ formic acid–1 ‰ formic acid, gradient elution	40	UV 270 nm	Zhou et al. (2010a)
Bibenzyls (1 chemical marker)	*D. aurantiacum* var. *denneanum*	Ultrasonic extraction with methonal	ODS (Shim-Pack CLC) (4.6 mm × 150 mm, 5 μm)	Acetonitrile–2 ‰ formic acid (35:65) Flow rate: 1.0 mL/min	25	UV 280 nm	Yang et al. (2007b)
Bibenzyls (1 chemical marker)	*D. chrysotoxum*	Ultrasonic extraction with methonal	XDB-C18 (Agilent Zorbax Eclipse) (4.6 mm × 250 mm, 5 μm)	Methanol–acetonitrile–water (30:30:40) Flow rate: 1.0 mL/min	18	UV 232 nm	Xia et al. (2008)

Table 3 continued

Analytes	Samples	Extraction method	Column	Mobile phase	Analytical time (min)	Detection	Refs.
Alkaloids (1 chemical marker)	*D. nobile*	Infiltration with aqueous ammonia and then refluxing extraction with chloroform	RP-18 (Waters XTerra^{TM}) (3.9 mm × 150 mm, 5 μm)	Acetonitrile–water–TEA (21:79:0.005) Flow rate: 1.0 mL/min	25	UV 210 nm	Li et al. (2009b)
Alkaloids (1 chemical marker)	*D. nobile*	Ultrasonic extraction with chloroform	XDB-C18 (Agilent ZORBAX) (4.6 mm × 250 mm, 5 μm)	Acetonitrile–phosphate buffer (pH = 8.0), gradient elution Flow rate: 0.8 mL/min	38	UV 198 nm	Xie (2008)
Coumarins and 2-glucosyloxy-cinnamic acids (6 chemical markers)	*D. thyrsiflorum*	Soak and then ultrasonic extraction with 50% (*v:v*) methonal aqueous solution	C18 (Polaris) (4.6 mm × 250 mm, 5 μm)	Acetonitrile–5 ‰ acetic acid, gradient elution Flow rate: 0.8 mL/min	45	UV 342 nm ESI–MS	Zhang et al. (2006a)
Coumarins (2 chemical markers)	*D. thyrsiflorum*	Ultrasonic extraction with methonal	ODS (Meta Chem) (4.6 mm × 250 mm, 5 μm)	Methanol–THF–1% acetic acid (15:5:80) Flow rate: 1.0 mL/min	20	UV 342 nm	Zhang et al. (2006b)
Coumarins (1 chemical marker)	8 *Dendrobium* species	Infiltration with aqueous ammonia and then refluxing extraction with chloroform	C18 (Kromasil) (4.6 mm × 250 mm, 5 μm)	Acetonitrile–water (20:80) Flow rate: 1.0 mL/min	25	UV 343 nm	Sun et al. (2008)
Coumarins (1 chemical marker)	*D. aurantiacum* var. *denneanum*	Ultrasonic extraction with methonal	C18 (Diamonsil) (4.6 mm × 200 mm, 5 μm)	Methonal–water (30:70) Flow rate: 1.0 mL/min	35	UV 274 nm	Zhou et al. (2010b)
2-Glucosyloxy-cinnamic acids (3 chemical markers)	3 *Dendrobium* species	Soak and then ultrasonic extraction with 50% (*v:v*) methonal aqueous solution	ODS-80Ts (Tosoh) (4.6 mm × 150 mm, 5 μm)	Methanol–5 ‰ formic acid, gradient elution Flow rate: 0.8 mL/min	50	UV 270 nm ESI–MS	Yang et al. (2007d)

TFA, trifluoroacetic acid; THF, tetrahydrofuran; TEA, triethylamine

Dendrobium, especially the sesquiterpenoid alkaloids, are extremely weak in ultraviolet absorption due to the absence of conjugated double bonds in their chemical structures. Thus, HPLC–UV methods for quantification of sesquiterpenoid alkaloids of *Dendrobium*, which are usually poorly sensitive, are actually inappropriate. Some other universal or sensitive approaches, such as evaporative light scattering detection (ELSD) or mass spectrometry, are suggested to be used to improve sensitivity. Additionally, comprehensive analysis, for example, simultaneous determination of multiple bioactive components by HPLC, is also desirable because the "holistic" actions of medicinal herbs are ascribed to complex chemicals. However, so far limited work has been done for the "holistic" quality control of *Dendrobium*.

Ultra-performance liquid chromatography (UPLC), utilizing sub-2 μm particles as solid phase and operating at much higher system pressure than that of HPLC, could perform analyses with higher resolution, greater sensitivity and greater speed with little solvent consumption. So UPLC has been more and more dominant in the area of pharmaceutical analysis, especially in the analysis of traditional Chinese medicines (Liang et al. 2009). Nevertheless, so far, only one study has reported on quantitative analysis for the quality control of *Dendrobium* by UPLC (Xu et al. 2010b), in which five components of *Dendrobium*, of three types, were baseline-separated within 6 min. With apparent superiority compared with HPLC with regard to resolution, sensitivity and analytical time, UPLC coupled with multiple detectors, such as UV and MS, should be widely used in qualitative and quantitative analysis for quality evaluation of *Dendrobium* in the future.

GC and TLC are also repeatedly used for quantitative purposes in quality control of *Dendrobium* (Cai et al. 2011; Wang and Zhao 1985). But their application is limited since GC is only available for volatile components while TLC quantification is relatively poor in reproducibility, resolution and sensitivity.

In short, numerous methods of qualitative and quantitative analysis for the quality assessment of *Dendrobium* have been developed. However, due to the extraordinary differences in morphological, microscopic, molecular and chemical characteristics of different *Dendrobium* herbs, establishing a universal approach for quality evaluation of multiple *Dendrobium* herbs remains difficult—a goal, not yet a reality.

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