· Reviews ·

Recent Advance in Chemical and Biological Studies on Cimicifugeae Pharmaceutical Resources

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Abstract: Cimicifugeae is one of the rich sources for various active components and the health promoting and therapeutic values of the components have been corroborated by long-term use in folk medicine and traditional Chinese medicine. Increasing interest in Cimicifugeae pharmaceutical resources has led to the further discoveries of triterpenoid saponins, phenolic compounds, chromones, and many other compounds in various species of Cimicifugeae, and to the investigations on their chemotaxonomy, molecular phylogeny, and bioactivities. Based on our pharmacophylogenetic studies, the progress in phytochemistry, chemotaxonomy, molecular biology, and phylogeny of Cimicifugeae had been summarized since 2007, especially *Cimicifuga* L. ex Wernisch. and *Actaea* L., and their relevance to therapeutic efficacy. An exhaustive literature survey is used to characterize the global scientific effort in the phytochemical and biological studies of Cimicifugeae. More triterpenoid saponins have been found in various species, among which the cimigenol type (type A) is predominant. The versatile bioactivities of saponins and extracts, as well as those of phenolics and other ingredients, were summarized and discussed. The morphology-based five-genus classification of Cimicifugeae is not supported by molecular phylogeny. Molecular phylogeny based on nuclear and chloroplast DNA sequences tends to merge *Cimicifuga* Wernisch., *Souliea* Franch., and *Actaea* L. into a single genus. It is indispensable to integrate the emerging technologies into Cimicifugeae research for both the sustainable utilization of Cimicifugeae pharmaceutical resources and finding novel compounds with potential clinical utility and less adverse effects. Systems biology and omics technologies would play an increasingly important role in booming pharmaceutical research involving bioactive compounds of Cimicifugeae.

Key words: biological activity; chemotaxonomy; Cimicifugeae; pharmaceutical resource; phylogeny; phytochemistry
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Introduction

Cimicifugeae plants, belonging to subfam. Helleboroideae and Ranunculaceae, are traditionally composed of five genera *Cimicifuga* L. ex Wernisch., *Actaea* L., *Anemonopsis* Siebold et Zucc., *Souliea* Franch., and *Beesia* Balf. f. et W. W. Sm. There are more than 40 species in the tribe Cimicifugeae, which are native to temperate regions of the Northern Hemisphere. The genus *Actaea* L. is closely related to *Cimicifuga* L. ex Wernisch. and *Souliea* Franch., and based on combined DNA sequence data and similarity in biochemical constituents and morphology, many botanists included those two genera within *Actaea* L. (Compton and Culham, 2002). If not merged, *Cimicifuga* L. ex Wernisch. has 28 species and eight are distributed in China. Among them, *Cimicifuga foetida* L., *C. dahurica* Maxim., and *C. heracleifolia* Komar. are officially listed in *China Pharmacopoeia 2010* as

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"*Shengma*", which have been used since ancient times to treat wind-heat headache, sore throat, toothache, and uterine prolapse, etc. Other species of *Cimicifuga* L. ex Wernisch. are also used in folk medicine to treat physical wounds, promote eruption, and detoxify (Gao *et al*, 2008). There are eight species in genus *Actaea* L., and six of them are distributed in North America. Among them, *Cimicifuga racemosa* (Nutt.) L. (CR), also called black cohosh, has long been used by native American to tackle rheumatism, menopausal, and nervous problems. Presently, black cohosh products are among the most popular health-promoting medicines in the US and Europe (Jiang *et al*, 2006). Two species of *Actaea* L. are used in traditional Chinese medicine and their therapeutic use is distinct from *Cimicifuga* Wernisch. (Gao *et al*, 2008). *Souliea* medicine is antipyretic and antitoxic, which is also used to eliminate dampness, clear heart fire, and relieve anxiety. *Beesia* Balf. f. et W. W. Sm. is endemic in China and is used to treat cold caused by exterior heat, joint pain of rheumatism, diarrhea, sore throat, headache, and snake bite. On the phylogenetic tree based on chloroplast and nuclear DNA sequences, *Beesia* Balf. f. et W. W. Smith. and the monotypic genus *Anemonopsis* Siebold et Zucc. together, and are basal to the cluster formed by *Cimicifuga* L. ex Wernisch., *Actaea* L., and *Souliea* Franch. (Wang *et al*, 2009). However, little is known about the chemical constituents and bioactivities of these two genera. *C. foetida* and *A. racemosa* are the most frequently studied. In this brief review, we summarized the recent progress in the phytochemical and biological studies of Cimicifugeae since 2007.

Triterpenoid saponins

The structures of rings A—D are not quite different in diverse cycloartane type saponins, whereas the side chains tied with ring D vary greatly, due to the difference in oxygenation and the approach of ring formation (Table 1 and Fig. 1).

Four new cycloartane compounds are structurally peculiar, namely, there is C-C bond cleavage between C-15 and C-16, as well as the six-membered lactone ring between C-15 and C-23 (Nian *et al*, 2012; Yoshimitsu, Nishida, and Nohara, 2007). Ring D of all

Table 1 Triterpenoid saponins found in species of Cimicifugeae since 2007

No.	Compounds	Aglycone	Species	Tissue	References
1	isocimipodocarpaside	L	*Cimicifuga racemosa*		Jamróz *et al*, 2012
2	3β,16α-dihydroxy-12-acetoxy-16,22-cyclo-23-ketone-24*R*,25-epoxy-cycloartane-7-ene 3-*O*-β-*D*-galactopyranoside	R	*C. simplex*		Kuang *et al*, 2012
3	24-*O*-hydroxy-7,8-didehydrohydroshengmanol 3-*O*-β-*D*-galactopyranoside	B			
4	24-*epi*-24-*O*-hydroxy-7,8-didehydrohydroshengmanol 3-*O*-β-*D*-galactopyranoside	B			
5	methyl 3,4-seco-4-hydroxy-3-cimigenolate	A	*C. foetida*	aerial parts	Nian *et al*, 2012
6	cimigenol-3-*O*-[2′,4′-*O*-diacetyl]-α-*L*-arabinopyranoside	A			
7	cimigenol-3-*O*-[3′,4′-*O*-diacetyl]-α-*L*-arabinopyranoside	A			
8	cimigenol-3-*O*-[4′-*O*-acetyl]-α-*L*-arabinopyranoside	A			
9	25-anhydrocimigenol-3-*O*-[3′-*O*-acetyl]-α-*L*-arabinopyranoside	A			
10	24-*epi*-cimigenol-3-one	A			
11	15,16-*seco*-7,8-didehydro-15-formyl-16-oxohydroshengmanol	P			
12	7,8-dihydro-11-dehydroxycimicidanol	D			
13	shengmanol-3-*O*-[2′-*O*-acetyl]-α-*L*-arabinopyranoside	B			
14	24-*epi*-cimigenol-3-one	A	*C. foetida*		Lu *et al*, 2012
15	foetinoside	M			
16	cimipodocarpaside	L	*C. racemosa*		Jamróz *et al*, 2011
17	shengmaxinsides A	A	*C. simplex*	root	Kuang *et al*, 2011
18	shengmaxinsides B	A			
19	shengmaxinsides C	B			
20	23-*O*-methyl-24-deoxy-2′-*O*-(3″-methylmalonyl)-cimiaceroside B	E	*C. foetida*	rhizomes	Sun *et al*, 2011

(To be continued)

(Continued Table 1)

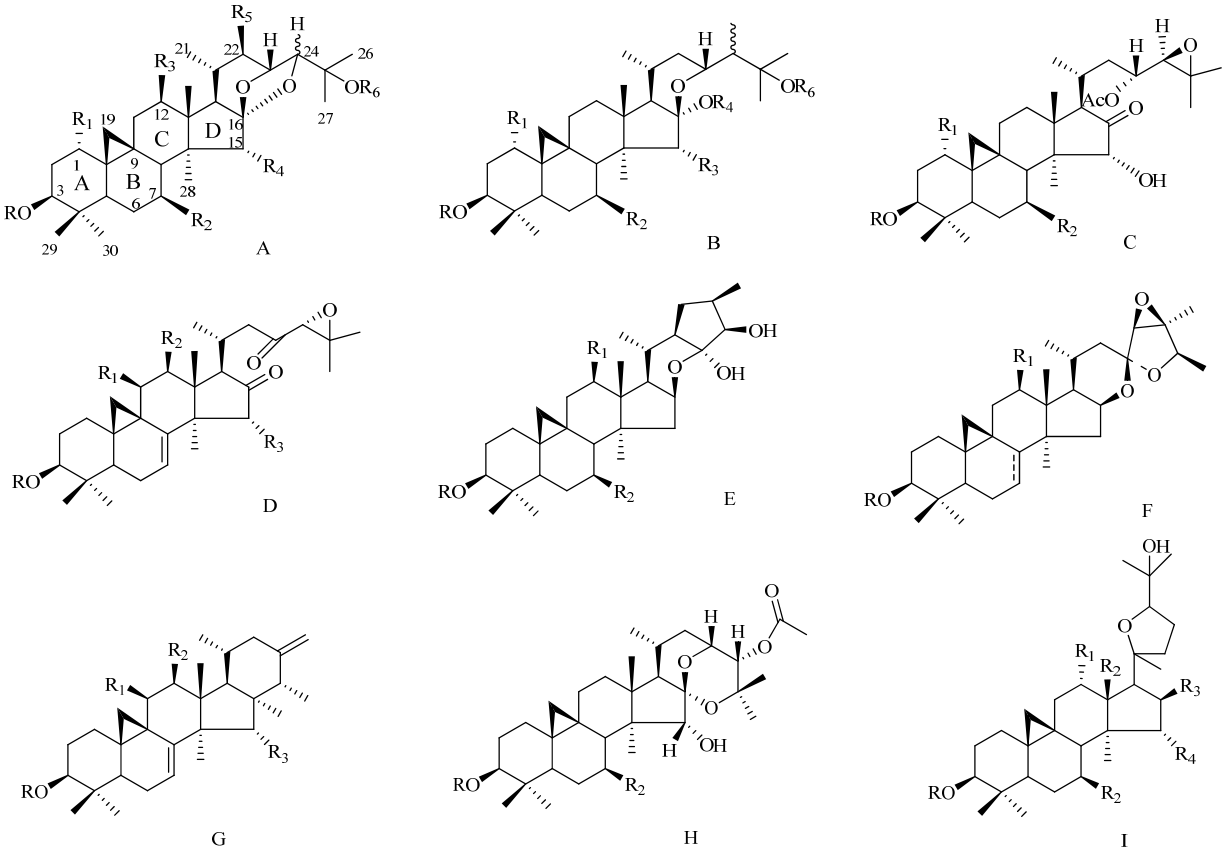
No.	Compounds	Aglycone	Species	Tissue	References
21	2′-*O*-(3″-methylmalonyl)actein	F			
22	2′,24-di-*O*-acetylisodahurinol-3-*O*-α-*L*-arabinopyranoside	B	*C. foetida*	aerial parts	Nian *et al*, 2011
23	24-*O*-acetylisodahurinol-3-*O*-α-*L*-arabinopyranoside	B			
24	12β-hydroxy-25-anhydrocimigenol	A			
25	cimigenol-12-one	A			
26	12β-hydroxy-15-deoxycimigenol	A			
27	2′-*O*-acetyl-24-epi-cimigenol-3-*O*-α-*L*-arabinopyranoside	A			
28	2′-*O*-acetylcimigenol-3-*O*-β-*D*-xylopyranoside	A			
29	25-anhydrocimigenol-3-*O*-α-*L*-arabinopyranoside	A			
30	2′,23-di-*O*-acetylshengmanol-3-*O*-α-*L*-arabinopyranoside	C			
31	2′,24-di-*O*-acetyl-25-anhydroshengmanol-3-*O*-α-*L*-arabinopyranoside	B			
32	3β,15α,16α,24α-tetrahydroxy-25,26,27-trinor-16,24-cyclo-cycloartane-23-one 3-*O*-β-*D*-xylopyranoside	G	*C. heracleifolia*	rhizomes	Nishida and Yoshimitsu, 2011
33	3β,15α,16α,-24α-tetrahydroxy-25,26,27-trinor-16,24-cyclo-cycloart-7-en-23-one 3-*O*-β-*D*-xylopyranoside	G			
34	12β-acetoxy-3β,15α,16α,-24α-tetrahydroxy-25,26,27-trinor-16,24-cyclo-cycloart-7-en-23-one 3-*O*-β-*D*-xylopyranoside	G			
35	3β,11β-dihydroxy-24,25,26,27-tetranor-cycloart-7-en-23, 16β-olide 3-*O*-β-*D*-xylopyranoside	O			
36	23*R*,24*S*-diacetoxy-3β,15α,25-trihydroxy-cycloart-7-en-16-one 3-*O*-β-*D*-xylopyranoside	M			
37	23*R*-acetoxy-3β,15α,24*R*,25-tetrahydroxy-cycloart-7-en-16-one 3-*O*-β-*D*-xylopyranoside	M			
38	foetidinosides A	S	*C. foetida*		Lu *et al*, 2010
39	foetidinosides B	L			
40	foetidinosides C	M			
41	foetidinosides D	M			
42	foetidinosides E	M			
43	3-*O*-β-*D*-xylopyranosyl cimigenol 15-*O*-β-*D*-glucopyranoside	A	*C. foetida*	rhizomes	Shen, 2010
44	25-*O*-acetyl cimigenol 3-*O*-β-*D*-xylopyranosyl 15-*O*-β-*D*-galactopyranoside	A			
45	cimifoetiside A		*C. foetida*	aerial parts	Pan *et al*, 2009; 2009b
46	cimifoetiside B				
47	15α-hydroxy-16-dehydroxy-16(24)-en-foetidinol-3-*O*-β-*D*-xylopyranoside	G	*C. foetida*	rhizomes	Lu *et al*, 2009
48	28-hydroxy-foetidinol-3-*O*-β-*D*-xylopyranoside	G			
49	foetidinol-3-*O*-β-*D*-xylopyranosyl-(1″→3′)-β-*D*-xylopyranoside	G			
50	(3′,12β)-*O*-diacetyl-cimigenol-3-*O*-β-*D*-xylopyranoside	A	*Actaea asiatica*	rhizomes	Fan *et al*, 2009
51	(4′,25)-*O*-diacetyl-cimigenol-3-*O*-β-*D*-xylopyranoside	A			
52	2′-*O*-acetyl-25-*O*-methyl-cimigenol-3-*O*-β-*D*-xylopyranoside	A			
53	2′-*O*-acetyl-25-*O*-ethyl-cimigenol-3-*O*-β-*D*-xylopyranoside	A			
54	3′-*O*-acetyl-cimicifugoside	F			
55	4′-*O*-acetyl-23-epi-26-deoxycimifugoside	F			
56	(23*R*)-26-deoxycimicifugoside	F	*A. asiatica*	rhizomes	Lu, Fan, and Duan, 2008
57	cimiaceroside C	E	*C. foetida*	rhizomes	Sun *et al*, 2008
58	cimifosides A	A			
59	cimifosides B	A			
60	cimifosides C	B			
61	cimifosides D	F			
62	7,8-dihydroactaeaepoxide 3-*O*-β-*D*-xylopyranoside	N	*A. pachypoda*	roots	Ali *et al*, 2007b

(To be continued)

(Continued Table 1)

No.	Compounds	Aglycone	Species	Tissue	References
63	12-deacetoxyactaeaepoxide 3-*O*-β-*D*-xylopyranoside	N			
64	12β-acetoxycimigenol	A			
65	podocarpasides A	L	*A. podocarpa*	roots	Ali *et al*, 2007a
66	podocarpasides B	L			
67	podocarpasides C	L			
68	podocarpasides D	L			
69	podocarpasides E	L			
70	podocarpasides F	L			
71	podocarpasides G	L			
72	podocarpasides H	D	*A. podocarpa*	roots	Ali, Khan, and Khan, 2007
73	podocarpasides I	D			
74	podocarpasides J	D			
75	24-acetoxy-15,16-*seco*-cycloartane 3-*O*-β-*D*-xylopyranoside	P	*Cimicifuga*	rhizomes	Yoshimitsu, Nishida, and Nohara , 2007
76	24-*epi*-hydro-15,16-*seco*-cycloartane 3-*O*-β-*D*-xylopyranoside	P			
77	24-*epimer*-hydro-15,16-*seco*-cycloartane 3-*O*-β-*D*-xylopyranoside	P			
78	cimifoetiside VI	B	*C. foetida*	aerial parts	Pan *et al*, 2007
79	cimifoetiside VII	B			
80	cimicifugadine	Q	*C. foetida*	roots	Dan *et al*, 2007
81	cimicifoetisides A	A	*C. foetida*	rhizomes	Sun *et al*, 2007
82	cimicifoetisides B	A			

A: cimigenol type B: hydroshengmanol type C: shengmanol type D: 16,23-dione type E: cimiacerogenin type F: cimifugenin type G: side chain-seco type H: neocimicigenoside type I: beesioside type J: asiaticoside type K: ranunculane type L: 9,10-*seco*-9,19-cycloartane type M: 9,19-cycloartane type N: actaeaepoxide type O: tetranor type P: 15,16-*seco*-cycloartane type Q: cycloartane triterpene alkaloid type R: 16,22-*seco*-cycloartane type S: lanostane type; same as below



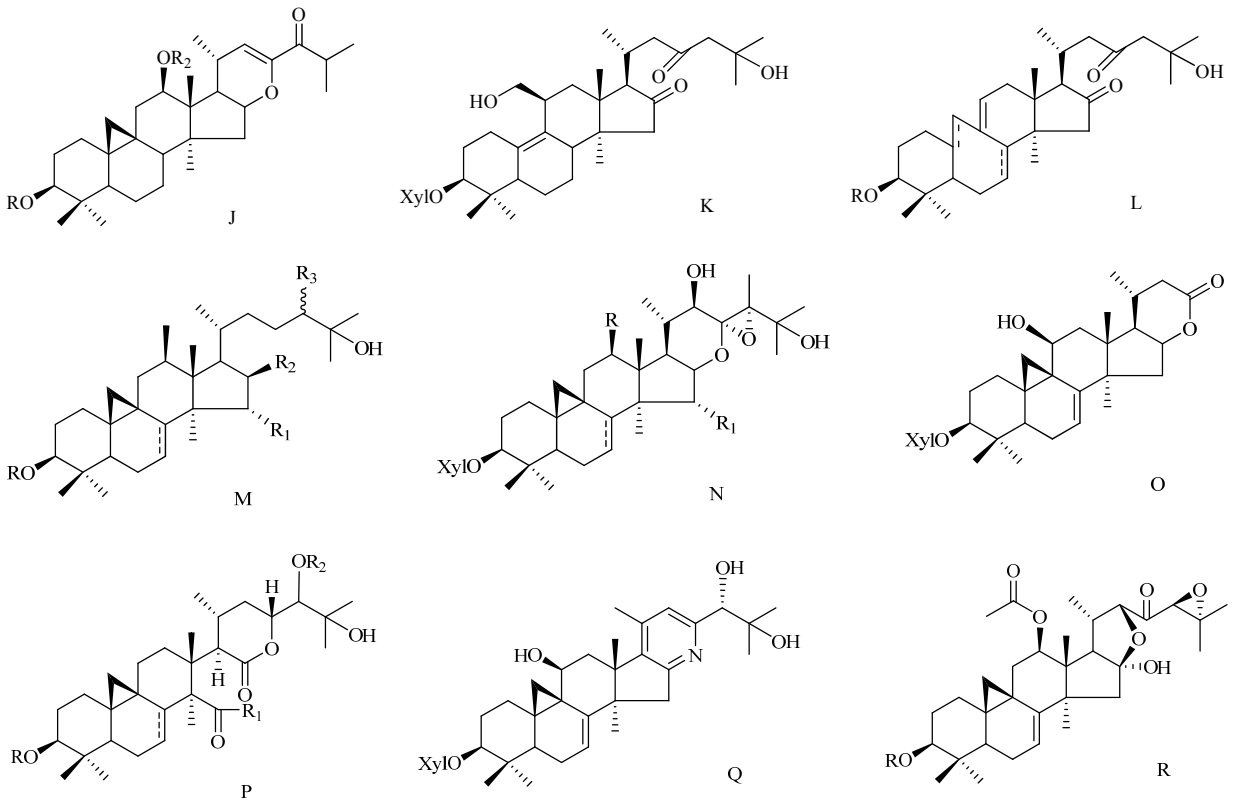


Fig. 1 Chemical structure types of 9,19-cycloartane triterpenoids in tribe Cimicifugeae

other cycloartane compounds is closed. An unprecedented triterpene alkaloid glycoside, designated cimicifugadine, with a pyridine ring incorporated into a cycloartane tirterpenoid nucleus, has been isolated from *C. foetida* (Dan *et al*, 2007). Several glycosides are characterized as 9,10-*seco*-9,19-cycloartane type with change in the cleavage between C-9 and C-10 (Jamróz *et al*, 2012; 2011; Lu *et al*, 2010; Ali *et al*, 2007a). C-3β is frequently glycosylated and usually linked with one or more sugars. The most common sugar is pentose (usually xylose and arabinose), while hexose (e.g., glucose and galactose) is occasional. Sometimes C-15, 16, 22, 24, and 25 are also glycosylated.

Bioactivities and adverse effects of saponins and extracts from Cimicifugeae

Anticancer activity

Novel anticancer compounds from natural product, due to their safety and efficiency, provide a gorgeous substitute to synthetic chemicals. Cimigenol from *C. foetida* exerted potent cytotoxic activity against SMMC-7721 (7.87 μmol/L) and A-549 (12.16 μmol/L), while cimiacerin B also showed the obvious cytotoxicity against A-549 cell line (Lu *et al*, 2012). The research group led by Prof. XIAO Pei-gen found that *C. foetida* extract inhibited the proliferation of hepatocellular cells via the induction of cell cycle arrest and apoptosis (Tian *et al*, 2007a). CR (black cohosh) reduced Ki-67 and cyclin D1 protein expression in fibroadenoma and may have the chemopreventive potential for breast cancer (Einbond *et al*, 2012). Cimiside E from *C. heracleifolia*, one of source plants of *C. foetida*, arrests cell cycle and induces the cell apoptosis in gastric cancer cells (Guo *et al*, 2009). Total glycosides from the aerial parts of *C. dahurica* Thurez Maxim. (TGC), another source plant of *C. foetida*, induced G_0/G_1 HepG2 cell cycle arrest at lower concentration and triggered G_2/M arrest and apoptosis at higher concentration (Tian *et al*, 2007b). An increase in the ratio of Bax/Bcl-2 was implicated in TGC-induced apoptosis. Besides, TGC dose-dependently inhibited the growth of the implanted H_{22} tumor in mouse, treatment with the triterpene glycoside actein induced a stress response and apoptosis in human breast cancer cells, suggesting that compounds from the species of *Cimicifuga* L. ex Wernisch. may be useful in

the prevention and treatment of breast cancer (Einbond *et al*, 2008). Gene expression profiling reveals the effects of CR on the estrogen receptor positive breast cancer cell line MCF-7 (Gaube *et al*, 2007). No estrogenic but antiproliferative and proapoptotic gene expression was shown for CR in MCF-7 cells at the transcriptional level. The effects may be caused by the activation of different pathways. The cycloartane glycosides and their aglycones could be identified as the active components in CR. The methanolic extract of CR also activated genes that enhanced apoptosis and repressed cell cycle genes in MDA-MB-453 human breast cancer cells (Einbond *et al*, 2007). The CR extract BNO 1055 inhibited the proliferation of human prostate cancer-derived LNCaP cells (Seidlová-Wuttke, Thelen, and Wuttke, 2006).

Three cycloartane triterpenoids from the aerial parts of *C. foetida* exhibited broad-spectrum and moderated cytotoxic activities (Nian *et al*, 2011). Human heat shock protein (Hsp) 27 was increased in various human cancer cells and exhibited cyto-protective activity that could affect tumorigenesis and the susceptibility of tumors to cancer therapy, but its expression at 2000 mg/L *C. foetida* extract was diminished (Soler *et al*, 2011). Three 9,19-cycloartane triterpene glycosides exhibited the significant cytotoxicity against human HepG2 cells (Nian *et al*, 2010). Cimicifugoside, a triterpenoid from *C. simplex*, is a specific nucleoside transport inhibitor that could display the potentiation of methotrexate cytotoxicity (Yawata *et al*, 2009). The triterpene glycoside actein activated stress- and statin-associated responses and was bioavailable in Sprague-Dawley rats (Einbond *et al*, 2009). Actein reduced free fatty acid and cholesterol content in the liver and inhibited the growth of HepG2 liver cancer cells.

Anemopsis californica (Nutt.) Hook. & Arn. showed *in vitro* anticancer activity against human colon cancer cells HCT-8 and the breast cancer cells (Kaminski *et al*, 2010). The growth inhibitory effect of *A. californica* in breast cancer cells was extracellular signal-regulated kinase (ERK)-mediated (Daniels *et al*, 2006). 25-Anhydrocimigenol-3-*O*-β-*D*-xylopyranoside isolated from *Souliea vaginata* (Maxim.) Franch. showed the anticancer activity against hepatoma, as it might induce apoptosis and G_0/G_1 cell cycle arrest (Tian *et al*, 2006). The anticancer activity of *Beesia* Balf. f. et W. W. Sm. awaits further studies. In the future, the structure-activity relationship of various saponins and their potentials in the treatment of various human cancers should be studied for novel drug development.

Effects on menopausal symptoms

CR has been used in Europe as a medicinal plant for more than a century and its roots have been widely used for the treatment of menopausal symptoms. Recently randomized studies have shown that CR consumption alleviated “hot flush” and due to the lack of uterotropic effects it could be a safe option for estrogen replacement therapy (Rachoń *et al*, 2008). The isopropanol extract of CR may act on the hypothalamic nuclei and have the therapeutic effects on menopausal symptoms (Zhang *et al*, 2012). Hot flush is a disorder of thermoregulation due to the lack of estrogens and is the most common and characteristic climacteric complaint. The CR special extract BNO 1055 could prevent hot flashes in ovariectomized rats (Kapur, Wuttke, and Seidlova-Wuttke, 2010). The combination of CR with *Hypericum perforatum* L. (HP) had a positive effect on climacteric complaints, while CR monotherapy as well as HP and *Vitex agnus-castus* L. was not better than placebo (Laakmann *et al*, 2012). A standardized isopropanolic CR extract (remifemin) was safe and effective for menopausal symptoms (Ross, 2012). It was an effective agent to manage Chinese women with climacteric symptom (Bai *et al*, 2009). It had the similar therapeutic effect and lower incidence of adverse effect when compared with Tibolone. For the CR extract, the onset of affecting abnormal thermoregulation took longer than that of estradiol valerate in ovariectomized rats (Ma *et al*, 2011b). CR extract had a significant effect on day central body temperature (CBT) but did not affect night CBT of ovariectomized rats. CR extract might be a reasonable treatment in tamoxifen-treated breast cancer patients with predominantly psychovegetative symptoms (Rostock *et al*, 2011). It was hypothesized that the established positive allosteric modulation of γ-aminobutryic acid (GABA) type A receptors mighr contribute to the beneficial effects of CR extracts in the treatment of climacteric symptoms (Cicek *et al*, 2010). Four cycloartane glycosides of CR significantly enhanced

GABA-induced chloride currents.

Osteoprotective effects

Triterpenoids from *Cimicifugae Rhizoma* are a novel class of inhibitors on bone resorption and ovariectomy-induced bone loss (Li *et al*, 2007). CR extract had osteoprotective effects and its triterpene saponins were responsible for the reduction of bone marrow fat and the secretion of pro-inflammatory cytokines (Seidlova-Wuttke *et al*, 2012a; 2012b). CR facilitated metaphyseal fracture healing in the early stage of osteoporosis in ovariectomized rats (Kolios *et al*, 2010). Deoxyactein isolated from CR protected osteoblastic MC3T3-E1 cells against antimycin A-induced cytotoxicity (Choi, 2011). Deoxyactein stimulated osteoblast function and inhibited bone-resorbing mediators in MC3T3-E1 cells (Choi, 2013). *C. heracleifolia* significantly preserved trabecular bone mass, bone volume, trabecular number, trabecular thickness, structure model index, and bone mineral density of proximal tibia metaphysis or distal femur metaphysis, thus preventing ovariectomy-induced bone loss in mice (Ahn *et al*, 2012). Cycloartane triterpenoids of *C. foetida*, such as cimigenol, actein, (23*R*,24*S*)25-*O*-acetyl-cimigenol-3-*O*-β-*D*-xylopyranoside, and (23*R*,24*S*)cimigenol-3-*O*-β-*D*-xylopyranoside, exhibited osteoclast inhibition activity (Dan *et al*, 2009). A triterpene glycoside from CR inhibited osteoclastogenesis by modulating tumor necrosis factor (TNF)-α and RANKL (a member of the TNF superfamily) signaling pathways (Qiu *et al*, 2007). The special extract of CR BNO 1055 was shown to have bone protective effects without exerting estrogenic effects in the uterus or mammary gland, as well as other organs that also express estrogen receptors (Seidlova-Wuttke, Jarry, and Wuttke, 2009).

Anti-inflammatory and immunosuppressive activities

Cycloartane-type triterpene glycosides from the rhizomes of *C. heracleifolia* and *C. foetida* showed anticomplement activity (Lee *et al*, 2012; Qiu *et al*, 2006). Cyclolanostane triterpene diglycosides from the aerial part of *C. foetida* effectively inhibited the proliferation of murine splenocytes induced by concanavalin A (Pan *et al*, 2009). A non-physiological accumulation of fat cells in abdomen and joints could result in the increased production of proinflammatory cytokines that have the adverse effects on serum lipids and glucose and on joint cartilage. The special extract of CR BNO 1055 was shown to reduce the size of the fat depot. This extract and its saponin prevented the metabolic syndrome and deterioration of cartilage in the knee joint of ovariectomized rats (Seidlova-Wuttke *et al*, 2012a; 2012b). Combined prescription of *Aralia cordata* Thunb. and *C. heracleifolia* and its major compounds inhibited matrix proteinases and vascular endothelial growth factor through the regulation of mitogen-activated protein kinase (MAPK) pathway, thus providing a therapy for osteoarthritis (Huh *et al*, 2011). Isoimperatorin, cimiside E, and 23-*O*-acetylshengmanol-3-xyloside from *Cimicifugae Rhizoma* inhibited TNF-α-induced vascular cell adhesion molecule (VCAM)-1 expression in human endothelial cells (Moon *et al*, 2011). Peroxisome proliferator-activated receptor-γ (PPAR-γ) upregulation and PI3K, ERK 1/2, and protein kinase C (PKC) signal pathways are involved in their anti-inflammatory activities. Cimiracemate A from multiple species of *Cimicifuga* L. ex Wernisch. suppressed the lipopolysaccharide-induced TNFα production in the blood macrophages (Yang *et al*, 2009). It might modulate the activities of signaling MAPK and transcription factor such as nuclear factor-κB (NF-κB).

Effects on cardiovascular system

CR relaxed the isolated rat thoracic aorta through endothelium-dependent and -independent mechanisms (Kim, Lee, and Rhyu, 2011). CR extract elicited the vasorelaxant effect via the NO/cGMP pathway. CR-induced endothelium-independent vasorelaxation appeared to involve the inhibition of calcium influx mediated by the opening of inward rectifier potassium channels. 7,8-Didehydrocimigenol (DHC), a triterpenoid of *Cimicifugae Rhizoma*, could increase the expression of PPAR-γ in endothelial cells (ECs) in a time- and dose-dependent manner (Mun *et al*, 2011). 7,8-DHC could inhibit TNF-α-induced expression of VCAM-1 but not ICAM-1 through upregulation of PPAR-γ in human ECs and could be used for the treatment of cardiovascular disorders such as atherosclerosis.

Other positive effects

Ig administration of CR extract attenuated psychological and physiological stress responses (Nadaoka *et al*, 2012a). The CR extract could alleviate

the acute stress responses by suppressing the changes of amine-to-metabolite ratio in brain (Nadaoka *et al*, 2012b). The CR extract significantly prevented the development of water immersion stress-induced gastric mucosal ulcers in rats (Nadaoka *et al*, 2012c).

CR extracts reduced endometrial proliferation in comparison to the placebo (Alves *et al*, 2008). CR decreased local estrogen formation in normal human breast tissue *in vitro*, which might contribute to the lack of hormonal effects of black cohosh in breast tissue observed in previous studies (Stute *et al*, 2007). Adding dry extract from CR rhizome to clomiphene citrate induction could improve the pregnancy rate and cycle outcomes in couples with unexplained infertility and recurrent clomiphene citrate induction failure (Shahin *et al*, 2008).

Dichloromethane fraction of *C. heracleifolia* decreased the level of melanin synthesis by activating the ERK or AKT signaling pathway in B16F10 cells (Jang *et al*, 2009). *C. heracleifolia* would be a useful therapeutic herb for treating hyperpigmentation and an effective component in whitening and/or lightening cosmetics.

Adverse effects and safety

Eight triterpene glycosides of CR were identified as competitive CYP (cytochrome P450) 3A4 inhibitors with IC_{50} values ranging from 2.3—5.1 μmol/L, while the alkaloids protopine and allocryptopine were identified as competitive CYP2D6 inhibitors (Li *et al*, 2011). Co-administration of CR with tamoxifen might interfere with the clinical efficacy of this drug. CYPs 1A2, 2D6, 2C9, 2C19, and 3A4 were inhibited *in vitro* by CR extracts (Huang *et al*, 2010; Ho *et al*, 2011). Fukinolic acid derivatives and triterpene glycosides from CR inhibited CYP isozymes, but were not cytotoxic to HepG2 cells *in vitro*. However, Pang *et al* (2011) suggested that the incidence of herb-drug interaction in patients administered with CR might not be mediated by human pregnane X receptor and CYP3A4. With the exceptions of St. John's wort and goldenseal, the currently available information suggested that concomitant intake of the herbal drugs, e.g., the well-known ginseng and CR, was not a major risk for drugs that were metabolized by CYPs (Zadoyan and Fuhr, 2012).

CR extract induced dose-dependent hematological changes, i.e., a non-regenerative macrocytic anemia, and increased the frequencies of peripheral micro-nucleated red blood cells in female B6C3F1/N mice and Wistar Han rats (Mercado-Feliciano *et al*, 2012). CR would not influence breast cancer risk if given to women before tumor formation. However, it increased metastatic mammary cancer in transgenic mice expressing c-erbB2 (Davis *et al*, 2008). CR impaired the fatty acid β-oxidation and induced oxidative stress in livers of ovariectomized rats with renovascular hypertension (Campos *et al*, 2012). The use of CR might not exert an explicit hepatotoxicity risk, but quality problems in a few CR products were evident that required additional regulatory quality specifications (Teschke *et al*, 2011).

Bioactivity of other compounds in tribe Cimicifugeae

Phenolic compounds

Petasiphenone, a phenol isolated from CR, inhibited the proliferation of the human prostate cancer cell line LNCaP *in vitro* (Jarry *et al*, 2007). The radical scavenging activity of the extracts from *Actaea* L. correlated to their polyphenolic composition (Nuntanakorn *et al*, 2007). Isoferulic acid (3-hydroxy-4-methoxycinnamic acid) from *Cimicifugae Rhizoma* is an effective natural anti-oxidant in both lipid and aqueous media (Wang, Li, and Chen, 2011). Actaealactone and a new phenylpropanoid ester derivative, cimicifugic acid G from CR, displayed the anti-oxidative activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical assay (Nuntanakorn *et al*, 2006).

Fukinolic acid and cimicifugic acids A—J, from a mixture of *C. dahurica* and *C. heracleifolia*, showed stronger hyaluronidase inhibitory activities than the positive control, rosmarinic acid (Iwanaga *et al*, 2010a). Cimicifugic acids K—N from *C. simplex* showed more potent hyaluronidase inhibitory activities than rosmarinic acid (Iwanaga *et al*, 2010b). Isoferulic acid was the major active principle in CR root extract, responsible for the observed inhibition of interleukin (IL)-6, TNF-α, and interferon-γ, but not for IL-8 stimulation (Schmid *et al*, 2009). The effect of this compound may explain the anti-inflammatory activities of CR and its beneficial actions in rheumatism and other inflammatory diseases.

Others

Cimicifugin from *C. foetida* inhibited human respiratory syncytial virus (HRSV; Wang *et al*, 2012a). *C. foetida* dose-dependently inhibited viral attachment and could increase heparin effect on viral attachment (Wang *et al*, 2012b). Additionally, *C. foetida* time- and dose-dependently inhibited HRSV internalization.

Serotonergic receptors and transporters are involved in thermoregulation. Various guanidine alkaloids and Pictet-Spengler adducts were detected in CR (Gödecke *et al*, 2009). These strongly basic and frequently zwitterionic nitrogenous metabolites contribute considerable chemical diversity to the polar serotonergic fraction of CR, which is used to alleviate menopausal symptoms. New phytochemical methods and liquid chromatography-mass spectrometry (LC-MS) led to the identification of *N*(ω)-methylserotonin as serotonergic active principle of CR (Gödecke *et al*, 2009). *N*(ω)-methylserotonin showed 5-hydroxytryptamine (serotonin) 7 receptor binding, induced cAMP and blocked serotonin re-uptake, suggesting that *N*(ω)-methylserotonin might be responsible for the serotonergic activity of CR (Powell *et al*, 2008).

The oils extracted from the roots of *A. californica* demonstrated the antiproliferative activity against AN3CA and HeLa cells *in vitro* (Medina-Holguín *et al*, 2008). Steam-distilled oil of *A. californica* had antimicrobial properties against three of 11 microbial species tested (Medina *et al*, 2005). This bioactivity could be partially accounted by the α-pinene in oil. A new 4α-methyl sterol from the aerial parts of *C. foetida*, cimisterol A, exhibited broad-spectrum and potent cytotoxic activities against human HL-60, Jurkat, K562, U937, HepG-2, and SGC-7091 cell lines (Nian *et al*, 2012). The bioactivities of Cimicifugeae alkaloids await further studies.

Chemotaxonomy and authentication of Cimicifugeae

Black cohosh (*A. racemosa* and *C. racemosa*) is ranked among the top-selling herbs in the US. There is a risk for the adulteration with the similar-looking *C. americana*, which grows in the same habitats of the eastern US. Other adulterants found in the current global market are the Asian *Cimicifuga* L. ex Wernisch. species *C. foetida*, *C. heracleifolia*, and *C. dahurica*. Three of the 11 black cohosh products were found to contain the marker compound—cimifugin but not cimiracemoside C, indicating that these products contain Asian *Actaea* L. instead of black cohosh (Jiang *et al*, 2006). One product contained both black cohosh and an Asian species of *Actaea* L. The chemotaxonomic distinctiveness of the HPLC fingerprints allowed the identification of ten species of *Cimicifuga* L. ex Wernisch., including three North American species and seven Asian species (He *et al*, 2006). The triterpene glycoside cimigenol-3-*O*-arabinoside, cimifugin, and cimifugin-3-*O*-glucoside were suitable species-specific markers for the distinction of CR from the other species of *Cimicifuga* L. ex Wernisch. A rapid and reliable high-performance thin-layer chromatographic (HPTLC) method was developed for the identification of CR and the detection of its most common contaminants by fingerprint profiles (Ankli, Reich, and Steiner, 2008). The HPLC and LC-MS fingerprints for polyphenols and triterpene glycosides revealed the distinct patterns that made CR clearly distinguishable from most other species of *Actaea* L. (Jiang *et al*, 2011). Cimifugin and cimiracemoside F were found to be important to distinguish CR from most Asian species of *Actaea* L. CR products continue to be one of the most popular botanical supplements in the US and world markets, and the correct identification for the different species of *Actaea* L. is a key step for the good manufacturing practice.

Fifteen chemical markers, including 3 cimifugin derivatives, 11 triterpene glycosides and 1 alkaloid, were identified with HPLC-TOF-ESI-MS technique and principal component analysis (PCA), and the 16 species of *Actaea* L. were divided into three groups, Asian (seven species), North American (eight species) and *A. racemosa* groups (Ma *et al*, 2011a). The adulteration of botanical supplements is a major problem that efficacy of the original product could be diminished and even toxicity could arise. Therefore, identification of marker compounds may help in the quality control and standardization of botanical supplements. Ultra performance liquid chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, which is faster and needs less solvent than HPLC. UPLC-UV/ELSD and UPLC-MS were successfully used to analyze the

different CR market products as well as to distinguish other two species of *Actaea* L. (Avula *et al*, 2009). Thin-layer chromatography (TLC) and combined TLC-bioluminescence (Bioluminex) are efficient, economical, and effective techniques which provided characteristic patterns and toxicity profiles for CR, *A. pachypoda* Ell. (white cohosh), *A. podocarpa* DC. (yellow cohosh), and other congeners (Verbitski *et al*, 2008). In addition to identification, the fingerprint method provided insight into chemical interconversion processes occurring between the diverse triterpene glycosides contained in Cimicifugeae.

Molecular phylogeny and genomics

Sequence analysis of nuclear internal transcribed spacer (ITS) and fluorescence melting curve analysis of LightCycler real-time polymerase chain reaction (PCR) products were used to authenticate *C. foetida* from four substitutes: *C. heracleifolia*, *C. dahurica*, *C. acerina*, and *C. simplex* Wormsk (Xue, Li, and Wang, 2009). This method was expensive and did not resolve the phylogenetic relationship within Cimicifugeae. We retrieved the ITS and chloroplast (cp) DNA sequences from NCBI GenBank and constructed the phylogenetic tree. On the ITS tree (Fig. 2), *Beesia* Balf. f. et W. W. Sm. and *Anemonopsis* Siebold et Zucc. are basal to the intermingled *Actaea* L., *Cimicifuga* L. ex Wernisch., and *Souliea* Franch. sequences. There are two major clades. One clade includes *C. foetida*, *C. yunnanensis* Hsiao, *C. brachycarpa* Hsiao, *C. kashmiriana* J. Compton et Hedd., and *C. europaea* Schipcz., which is supported by the cp *trnL-F* tree; In the other clade, *C. heracleifolia* and *C. dahurica* are basal to other sequences, followed sequentially by *Souliea* Franch. and *C. nanchuanensis* Hsiao, an endemic endangered species in Sichuan, China. *C. acerina*, used as a folk medicine, grouped with two Japanese species, which is supported by the cp *matK* tree (not shown), while different *C. simplex* sequences cluster with distinct group. On the *trnL-F* tree, the group containing *C. foetida* and *C. yunnanensis* is closer to *C. heracleifolia* and *C. dahurica*, whereas *C. simplex* is closer to these Asian species. *C. yunnanensis* clusters with *C. foetida* (alternative names *C. mairei* and *C. frigida*) on both ITS and cp trees, implying that it might not be an independent species. *A. erythrocarpa* (Fisch.) Kom. and *A. asiatica* Hara of China are close to *A. rubra* (Ait.) Willd. and *A. spicata* L. of North America respectively, and *A. racemosa* (black cohosh) is closer to these species than to other taxa. The reciprocal position of these American species and *Shengma* source plants is inversed on the ITS and cp trees. The phylogenetic position of *Souliea* Franch. varies greatly. These observations implied the extensive hybridization of the ancestral taxa during Cimicifugeae evolution.

A. racemosa cDNA libraries were constructed from young leaf, rhizome, and root tissues (Spiering *et al*, 2011). Expressed sequence tags (ESTs) sequencing was performed and 1590 unigenes were assembled. Seventy putative secondary metabolism genes were identified, including 2, 3 oxidosqualene cyclase, BAHD-type acyltransferase, and tryptophan decarboxylase. This preliminary transcriptome study provided the initial insight into gene content and diversity in black cohosh, and generated the tools and resources for the detailed investigations of secondary metabolite genes and enzymes in this important medicinal plant. Seven microsatellite markers were found for *A. racemosa* (Pate *et al*, 2012). Most of the loci cross-amplified in *A. pachypoda* Elliot, *A. podocarpa* DC., and *A. rubra*, indicating the utility of these markers for the genus. In the future, more microsatellite markers could be mined from the transcriptome and genomic survey datasets (Hao *et al*, 2011; 2012), which will provide the tools for population genetic studies and molecular breeding.

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.512 682 92 was shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and were in the units of the number of base substitutions per site. The rate variation among sites was modeled with a γ distribution (shape parameter = 6.18). The differences in the composition bias among sequences were considered in evolutionary comparisons.

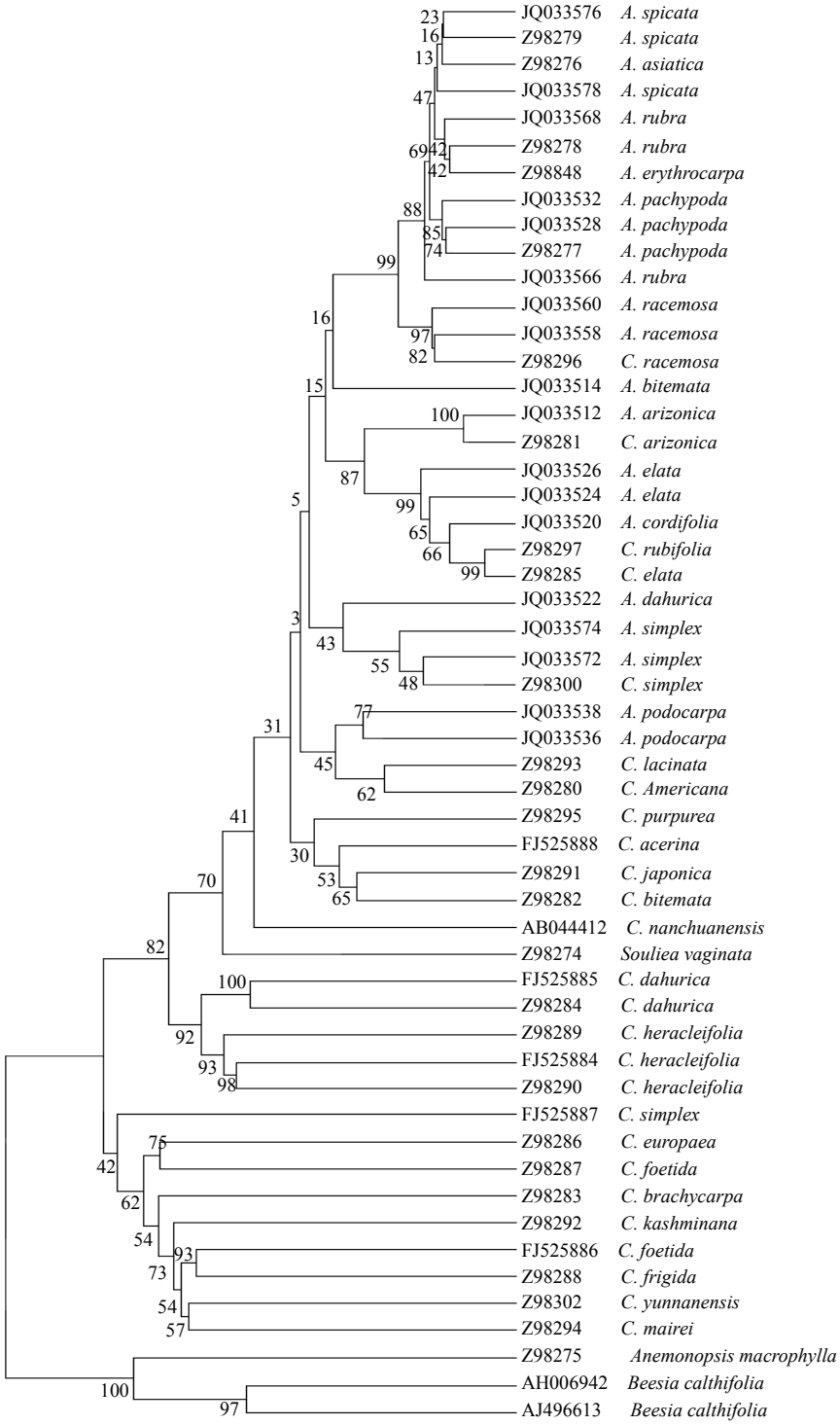


Fig. 2 Evolutionary relationships of Cimicifugeae ITS sequences

The analysis involved 53 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 754 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al*, 2011). *C. foetida*, *C. dahurica*, and *C. heracleifolia* are officially listed in *China Pharmacopoeia* as "*Shengma*".

Conclusion

The significant advances in the studies on pharmaceutical components, biological activities, taxonomy, and phylogeny of Cimicifugeae plants are summarized in this review. Recently more triterpenoid saponins have been found in Cimicifugeae plants, but their biosynthetic pathway has not been explored, which

hinders the cell-based production of useful secondary metabolites. Other secondary metabolites of Cimicifugeae plants are less studied and thus might be intriguing the topics in the future. Metabolomic and genomic studies of Cimicifugeae plants are just in their infancy. Various omics techniques should be put into full play in the drug research and development of Cimicifugeae.

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