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Mining chemodiversity from biodiversity: pharmacophylogeny of medicinal plants of *Ranunculaceae*

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[ABSTRACT] This paper reports a pharmacophylogenetic study of a medicinal plant family, *Ranunculaceae*, investigating the correlations between their phylogeny, chemical constituents, and pharmaceutical properties. Phytochemical, ethnopharmacological, and pharmacological data were integrated in the context of the systematics and molecular phylogeny of the *Ranunculaceae*. The chemical components of this family included several representative metabolic groups: benzylisoquinoline alkaloids, ranunculin, triterpenoid saponin, and diterpene alkaloids, among others. Ranunculin and magnoflorine were found to coexist in some genera. The pharmacophylogenetic analysis, integrated with therapeutic information, agreed with the taxonomy proposed previously, in which the family *Ranunculaceae* was divided into five sub-families: Ranunculoideae, Thalictroideae, Coptidoideae, Hydrastidoideae, and Glaucidioideae. It was plausible to organize the sub-family Ranunculoideae into ten tribes. The chemical constituents and therapeutic efficacy of each taxonomic group were reviewed, revealing the underlying connections between phylogeny, chemical diversity, and clinical use, which should facilitate the conservation and sustainable utilization of the pharmaceutical resources derived from the *Ranunculaceae*.

[KEY WORDS] *Ranunculaceae*; Pharmacophylogeny; Chemodiversity; Ethnopharmacology; Bioactivity; Sustainability

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Introduction

The plant family *Ranunculaceae* (eudicot Ranunculales), has about 60 genera and 2 200 species, consisting mostly of herbs such as small shrubs and woody vines, Plants of this family are distributed worldwide, mainly in the temperate regions of the northern hemisphere. Forty-two genera and about 720 species are distributed throughout China, most of which are found in the southwest mountainous region [1]. The morphological feature of this family is the primitive character, poly-carpellary. Plants of this family contain a broad variety of chemical components. At least 30 genera and about 220 species have medicinal uses in China, among which rhizoma coptidis, monkshood, rhizoma cimicifugae, and caulis clematidis armandii have a long history of use in traditional Chinese medicine [2]. *Ranunculaceae* plants have a complicated chemical composition, which typically has taxonomic implications [2]. There are numerous reports concerning the plant systematics, phytochemistry, chemotaxonomy, and pharmacology of this family [2]. With the development of the chemical sciences and analytical technologies, a large number of new chemical components and bioactivities, as well as therapeutic uses, have been found, providing new chemotaxonomic evidence for therapeutic implications. In this report, we attempted to provide a comprehensive review of relevant research in recent years, emphasizing the correlations between their phylogeny, chemical constituents, and pharmaceutical properties, *i.e.,* the pharmaphylogeny, of the plant family Ranunculaceae.

Systematics of Ranunculaceae

Tamura (1993) recognized five sub-families in the *Ranunculaceae* (Hydrastidoideae, Thalictroideae, Isopyroideae, Ranunculoideae, and Helleboroideae), mainly based on the chromosome and floral characteristics [3]. But Takhtajan has excluded the genera *Hydrastis* and *Glaucidium* from the family *Ranunculaceae* [4]. Peng *et al*. have agreed with the taxonomy

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proposed by Tamura, and divided the *Ranunculaceae* into six sub-families [5], including Helleboroideae, Ranunculoideae, Cimicifugoideae, Isopyroideae, Thalictroideae, and Coptidoideae. Based on a comprehensive analysis of the phylogeny, chemotaxonomy, ethnopharmacology, and demonstrated bioactivities, it seems plausible to treat the Cimicifugoideae as a separate sub-family.

The cladogram (Fig. 1) proposed by the APG II system and based on molecular phylogeny [6] consists of five sub-families, the basal Glaucidiaceae and Hydrastidoideae, the Coptoideae, and the evolutionarily young Thalictroideae and Ranunculoideae. Wang *et al*. have presented an updated classification based on four molecular loci and 65 morphological characters [7], including the cytology data and four chemotaxonomic markers. This classification agrees with the APG II cladogram and recognizes ten tribes within the sub-family Ranunculoideae (Fig. 1). The subfamily Thalictroideae includes genera, such as *Isopyrum*, *Dichocarpum* and *Aquilegia*, which belong to the previously proposed sub-family Isopyroideae [8]. Herein, the pharmacophylogeny discussed in this report is based on this taxonomic system.

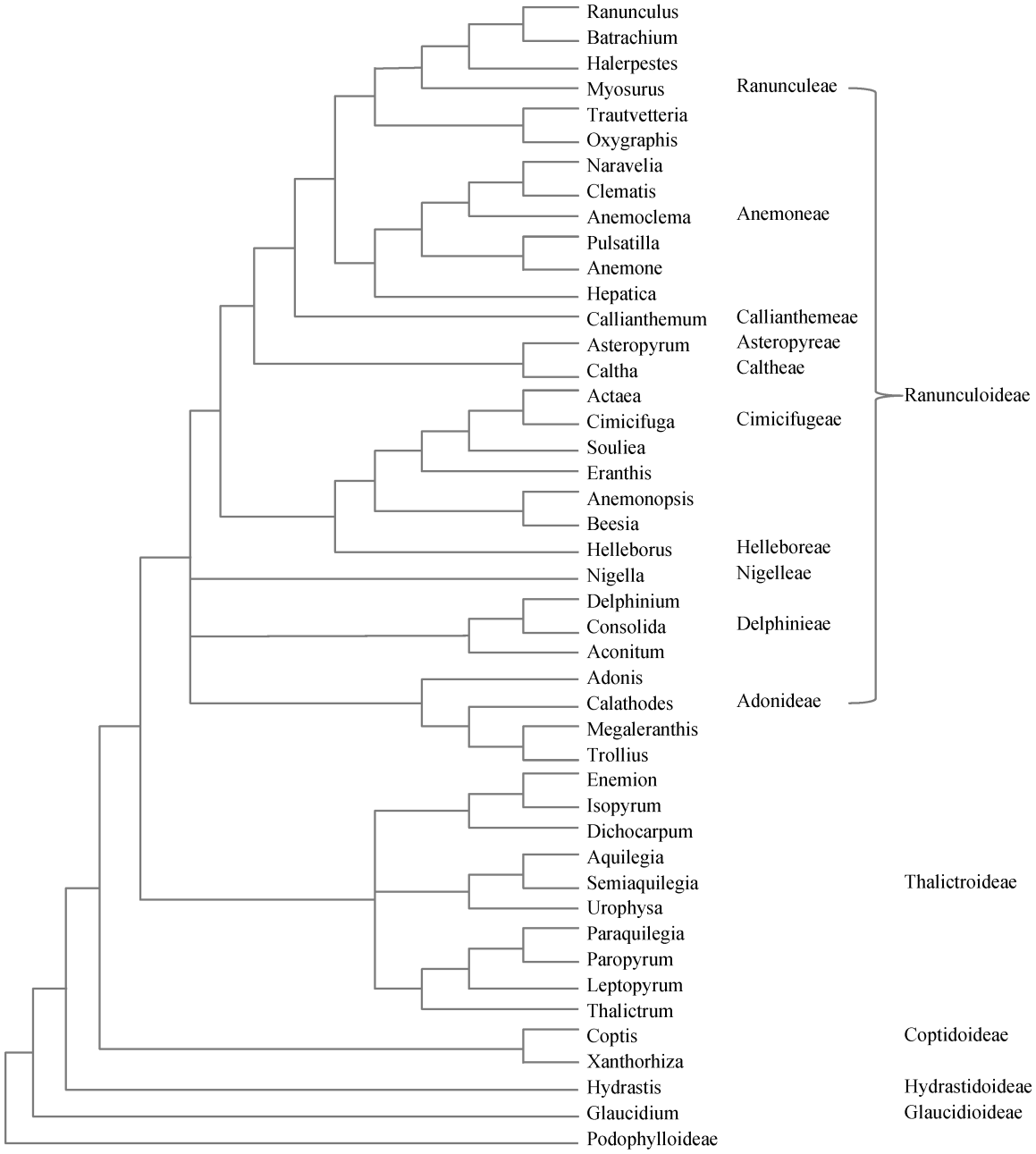

Fig. 1 Ranunculaceae cladogram according to the APG II system [6], modified from ref [7]

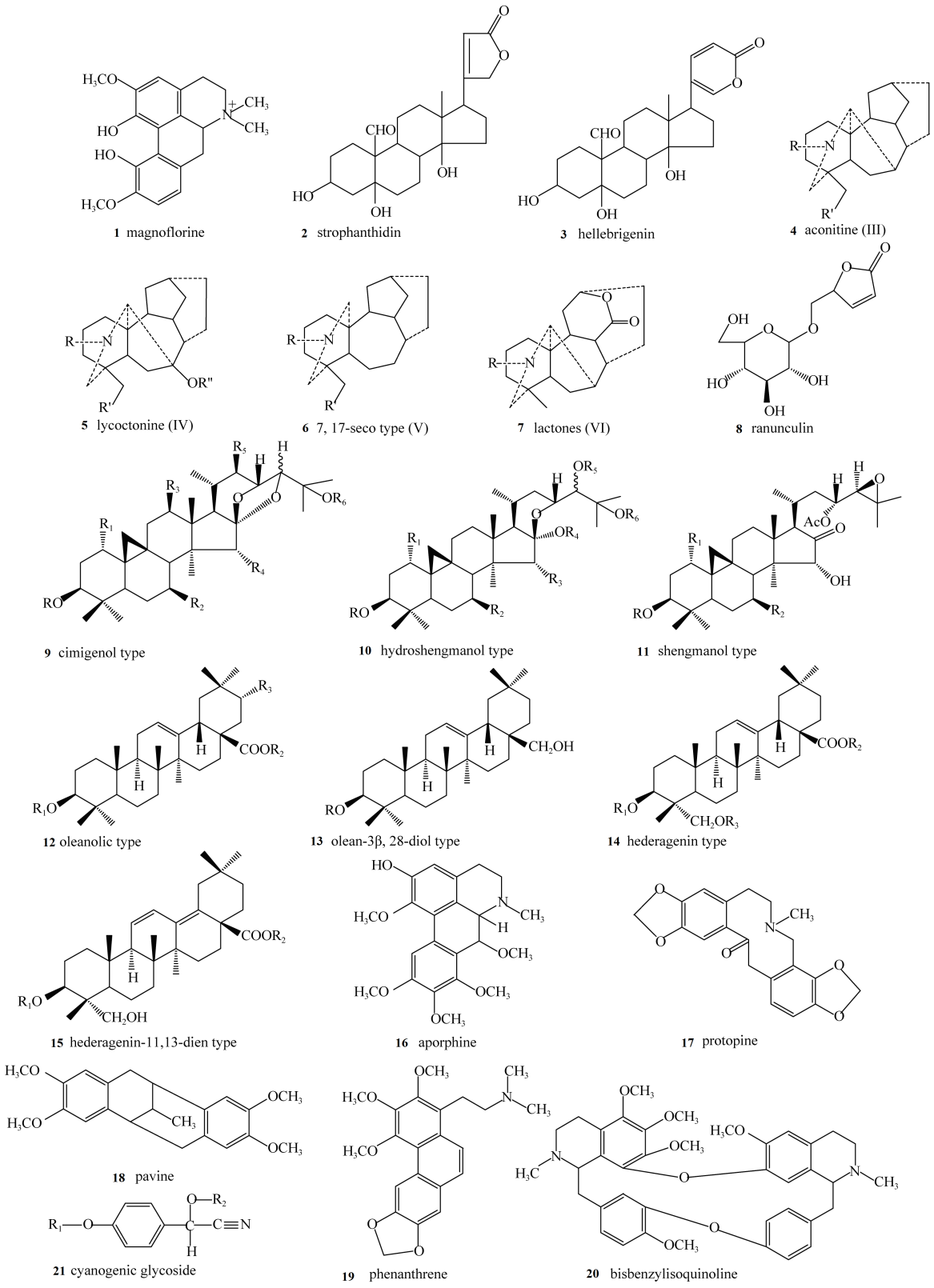

Fig. 2 Representative medicinal compounds found in the Ranunculaceae. 1. magnoflorine. cardiac glycoside; 2. strophanthidin; 3. hellebrigenin. diterpenoid alkaloid; 4. aconitine (III); 5. lycoctonine (IV); 6. 7, 17-seco type (V); 7. lactones (VI); 8. ranunculin. tetracyclic triterpenoid saponin; 9. cimigenol type; 10. hydroshengmanol type; 11. shengmanol type. pentacyclic triterpenoid saponin; 12. oleanolic type; 13. olean-3*β*, 28-diol type; 14. hederagenin type; 15. hederagenin-11, 13-dien type. benzylisoquinoline alkaloid; 16. aporphine; 17. protopine; 18. pavine; 19. phenanthrene; 20. bisbenzylisoquinoline; and 21. cyanogenic glycoside

The chemical composition of Ranunculoideae plants

Adonideae

The tribe is divided into four genera *Adonis*, *Calathodes*, *Trollius*, and *Megaleranthis*. *Adonis* is basal to the other genera. Magnoflorine (**1**, Fig. 2) and cardiac glycosides (*e.g.*, **2** and **3**, Fig. 2) are abundant in *Adonis* [2, 9]. Flavonoids and lactones are also found in *Adonis* [10]. Flavonoids are abundant in *Trollius*, and significantly more flavone *C*-glycosides are found than *O*-glycosides. The main types of alkaloidsare the pyrrolizidine alkaloids, such as senecionine, squalidine, and trolline. The known organic acids are globeflowery acid, palmitic acid, veratric acid, and proglobeflowery acid. The representative volatile organic compounds are sesquiterpenoids, fatty acid derivatives, and nitrogen-containing compounds [11]. Labdane type diterpenoids [12] and phenylethanoid glycosides [13] are also found in *Trollius*.

Delphinieae

In this tribe, the genus *Delphinium* is more closely related to the genus *Consolida* than to the genus *Aconitum*. This tribe is rich in diterpenoid alkaloids and flavonoids [14]. More C_{19}- and C_{20}-diterpenoid alkaloids are found than the C_{18}- alkaloids [15]. The oils from *Delphinium* seeds consist mainly of octadecadienoic (linoleic), hexadecanoic (palmitic), and octadecenoic (oleic) acids [16]. In *Consolida*, lycoctonine type (C_{19} type IV) and denudatine and hetisine types (C_{20} type VIII and X) are the known diterpenoid alkaloids [15]. Octadecenoic and hexadecanoic acids are the main essential oil constituents [16].

Diterpenoid alkaloids, polysaccharides, flavonoids, and phenolic acids are abundant in *Aconitum* species [15]. The aconitine type (C_{19} type III) is the predominant diterpenoid alkaloid (**4**, Fig. 2). Flavonols, *e.g.*, kaempferol and quercetin derivatives, are also prevalent in *Aconitum* species.

Nigelleae

The genus *Nigella* contains triterpenoid saponins and magnoflorine and other benzylisoquinoline alkaloids. Norditerpenoid alkaloids and pyrroloquinoline alkaloids are also found [17]. Thymoquinone, fatty acids and monoterpene hydrocarbons are the main components of *Nigella* essential oil [16, 18].

Helleboreae

Helleborus species contain magnoflorine and other benzylisoquinoline alkaloids, tetracyclic, and pentacyclic triterpenoid saponins, ranunculin (**8**, Fig. 2) and cardiac glycosides. Lactones, such as protoanemonin and bufadienolides [19], are abundant. Flavonoids, *β*-ecdysone, furostanol, and spirostanol steroidal saponins are also found [20-21].

Cimicifugeae

There are two main clades in this tribe. The genera *Anemonopsis* and *Beesia* are basal to other four genera, among which *Cimicifuga* is closer to *Actaea* than to *Souliea* or *Eranthis*. *Cimicifuga* and *Actaea* contain cycloartane triterpenoid glycosides, other tetracyclic triterpenoid saponins, and phenylpropanoids [22]. *Souliea* contains the soulieosides A-E (cycloartane-type), prim-*O*-glucosylcimifugin, and the alkaloid soulieotine. *Eranthis* contains magnoflorine and triterpenoid saponins [23]. *Beesia* contains cycloartane saponin beesiosides I-V and A-P. Cimigenol type (type A) is the predominant cimicifugeae triterpenoid saponin (**9**, Fig. 2). Phenolic compounds and chromones are also found in this tribe.

Caltheae

The genus *Caltha* contains the oleanane-type saponin polypetalosides A-I, and the triterpenoid lactones caltholide, epicaltholide, and protoanemonin. In *Caltha* spp. Sesquiterpenoids, fatty acid derivatives (*e.g.*, protoanemonin) and monoterpenoids are the dominating metabolites in the flower scent [11].

Asteropyreae

The water-soluble quaternary ammonium-type alkaloids, such as magnoflorine, palmatine, columbamine, and jatrorrhizine, are found in the genus *Asteropyrum* [24]. Berberine and berberrabine are also present [25].

Callianthemeae

Ranunculin is found in this tribe [5]. Flavonol glycosides and other phenolic compounds are also present in the genus *Callianthemum* [26].

Anemoneae

In this tribe, *Hepatica* is the basal genus, followed by the genera *Pulsatilla* and *Anemone*. The genus *Clematis* is closer to *Naravelia* than to *Anemoclema*. Identified *Anemone* compounds include triterpenoids, steroids, lactones, fats and oils, saccharides, and alkaloids [27]. Oleanolic acid triterpene saponin is abundant in *Anemone* species. *Anemone* contains ranunculin, anemonin, and protoanemonin, which are characteristic constituents of *Pulsatilla* and illustrate the close relationship between these two genera. *Anemone* also contains coumarins and flavonoids. Benzenoids, fatty acid derivatives (*e.g.*, pentadecane and nonanal), and sesquiterpenoids are the dominant volatile compounds in *Anemone* [11].

Anemonin, okinalin, and okinalein are abundant in the genus *Pulsatilla*, which also contains lupane and oleanane type pentacyclic triterpene saponins, lignans, and daucosterol. (+)-Pinoresinol, 13-peltatin, peltatin, and triterpenoic acids (*e.g.*, anemonic acid) are also found. Fatty acid derivatives (*e.g.*, protoanemonin and pentadecane) and monoterpenoids are the dominant volatile compounds in *Pulsatilla* [11].

Clematis contains saponin, coumarins, flavonoids, anthocyanins, and alkaloids [28]. Lignans, steroids, macrocyclic compounds, phenolic glycosides, and volatile oils are also found [29]. The common aglycones belong to the pentacyclic triterpene group such as oleanolic acid, hederagenin, and epihederagenin (**12**–**15**, Fig. 2). The representative coumarins are scopoletin, umbelliferone, and clemastanin A and B. Aporphinoid alkaloids and diterpenoid alkaloids, such as magnoflorine, corytuberine, elatine, and aconitine, are found. Ranunculin, anemonin and protoanemonin are also present in this genus.

Anemoclema contains the saponins anemoclemosides A and B [30]. *Naravelia* contains simple benzamide drivatives [31].

Ranunculeae

In this tribe, the genera *Oxygraphis* and *Trautvetteria* are basal to the other four genera, while *Ranunculus* is closer to *Batrachium* than to *Halerpestes*. Lactones, including protoanemonin, anemonin, ranunculin, isoranunculin, and ternatolide, are widely distributed in *Ranunculus* species. The aglycone of the triterpene saponins is hederagenin or oleanolic acid. Alkaloids are abundant in the genus *Ranunculus*, most of which are of the berberine-type and aporphinoid alkaloids. The dominant volatile compound in *Ranunculus* species is protoanemonin [11].

The chemical composition of Thalictroideae, Coptidoideae, Hydrastidoideae, and Glaucidioideae

Thalictrum clade

In this group, the genus *Thalictrum* is basal to the three other genera. The genus *Paraquilegia* contains magnoflorine and bisbenzylisoquinoline alkaloids, such as fangchinoline and dimethyltubocurarine. One of the characteristics of this genus is cyanide-containing compounds. Flavone *C*-glycosides and triterpene saponins are also present [32-33].

The genus *Thalictrum* has alkaloids, saponins, and flavonoids. More than 300 alkaloids have been found in this genus (*e.g.*, **16**–**20**, Fig. 2), many of which are bisbenzylisoquinoline and aporphinoid alkaloids. The atisine and hetidine type (C_{20} type VII and IX) diterpenoid alkaloids are found in this genus [15], which also contains tetracyclic and pentacyclic triterpenoid saponins.

Table 1 Distributions of *Ranunculaceae* compounds detected by TLC

Taxon	Alkaloids			Ranunculin	Triterpenes		Cardiac glycosides	Cyanogenic glycosides
	Diterpene alkaloid	Magnoflorine	Other isoquinoline alkaloids		Tetracyclic	Pentacyclic		
Ranunculoideae								
Ranunculeae								
Ranunculus		–	–	++		+		+
Batrachium		–	–	+				
Anemoneae								
Clematis		–	–	++		++		
Pulsatilla		–	–	+		+		
Anemone		–	–	++		++		
Hepatica		–	–	+				
Callianthemeae			–	–				
Asteropyreae		+	++	–				
Caltheae		+	+	–				
Cimicifugeae								
Actaea		–	–	–	+			
Cimicifuga		–	–	–	+			
Eranthis		–		–				
Helleboreae		–		+			+	
Nigelleae		+		–				
Delphinieae								
Delphinium	++	+		–				
Consolida	+	+		–				
Aconitum	++	+	+	+*				
Adonideae								
Adonis		+		–			++	
Trollius		+		–				
Thalictroideae								
Isopyrum		+	+	–				+
Aquilegia		+	+	–				+
Semiaquilegia		+		–				+
Leptopyrum		+		–				+
Thalictrum		+	++	–				+
Coptidoideae		+	++	–				

+, detected by thin layer chromatography (TLC); ++, more abundant than "+"; –, not detected by TLC; *, found in the root of *A. scaposum* var. *vaginatum* from Sichuan, China.

Aquilegia clade

In this group, the genus *Aquilegia* is closer to *Semiaquilegia* than to the genus *Urophysa*. *Aquilegia* contains flavonoids, phenolic acids, triterpenoid saponins (cycloartane glycosides), and alkaloids (magnoflorine and aporphinoid-type alkaloids) [34-36]. Labdane diterpene glycosides and megastigmane glycoside are also found [37]. The dominant volatile compounds of *Aquilegia* are fatty acid derivatives (*e.g.*, octanal, protoanemonin, and nonanal), and benzenoids [11]. Lactones, magnoflorine, phenolic glycosides, cyanogenic glycosides (**21**, Fig. 2), diterpenoids, and benzoic acid derivatives are found in the *Semiaquilegia* species [38-41].

Isopyrum clade

In this clade, *Isopyrum* is closer to *Enemion* than to *Dichocarpum*. *Isopyrum* is rich in bisbenzylisoquinoline alkaloids [2], *e.g.*, berbamine, tetrandrine and penduline, and other benzylisoquinoline alkaloids, such as magnoflorine, corytuberine and coptisine. *Isopyrum* contains flavonoids.

Coptidoideae

The genus *Coptis* contains protoberberine type alkaloids [2], such as berberine, palmatine, coptisine, and jatrorrhizine, which are quaternary ammonium type alkaloids. Magnoflorine is an aporphinoid alkaloid.

Hydrastidoideae

The major alkaloids in *Hydrastis* species are berberine, palmatine, hydrastine, hydrastinine, and canadine [42].

Glaucidioideae

Glaupalol-related coumarins are present in *Glaucidium palmatum* [43].

Ethnopharmacology and bioactivity

Different taxonomic groups have characteristic bioactivities and ethnopharmacological uses, depending on their different chemical profiles. During the past decades, our group has collected more than 30 000 cards that record the ethnopharmacological use of more than 5 000 Chinese medicinal plant species. The Traditional Remedy Index (*TRI*) is calculated by the equation $TRI = C_1^2/C_2 \times 100$ [44], where C_1 is the number of cards on which a specific ethnopharmacological use is recorded in China for a single genus; C_2 is the number of species that has the same ethnopharmacological use in the same genus. $TRI \geq 300$ is considered as significant. The higher the *TRI*, the more credible that the genus has the specific ethnopharmacological use. Distribution density of ethnopharmacological use (β) is calculated by theequation $\beta = SP_1/SP_2 \times 100$, where $SP_1 = C_2$, SP_2 = number of species in China in this genus.

Adonideae

The genus *Adonis* is used in various indications, such as heat-clearing and damp-drying (*TRI* 500, β 14), cardiac insufficiency (*TRI* 533, β 42), arrhythmia (*TRI* 514, β 28), dysentery (*TRI* 320, β 14), ulcer disease and sore (*TRI* 500, β 14) (Table 2), conjunctivitis (*TRI* 320, β 14), and vomiting (*TRI* 320, β 14). Cardenolide glycosides, strophanthidin glycoside, and pregnane glycosides, cymarin, and cymarol, have anti-cancer activity [45-47] (Table 3). Cymarilic acid shows antiangiogenic activity [47].

Trollius species have antibacterial effects and are used in the treatment of tonsillitis (*TRI* 514, β 42), otitis media (*TRI* 514, β 42), red eye with swelling and pain (*TRI* 450, β 57), and scrofula (*TRI* 400, β 57). Flavonoids and phenolics are the major anti- inflammatory and antioxidant components [48-49]. Flavone glycosides have antiviral [50], anti-complement [51], and DNA-binding activities [52]. Organic acids from *Trollius* show antiviral activities [53]. A *C*-glycosyl-flavone is a defense molecule against developing larvae [54]. The whole plant of *Calathodes* is used in rheumatic disease and numbness [2].

Delphinieae

Delphinium species are used in wind-dispelling and damp-drying (*TRI* 750, β 20), interior-warming and cold-dissipating (*TRI* 756, β 17), and the treatment of pesticide poisoning (*TRI* 1 056, β 27), arthritis (*TRI* 1 031, β 20), arthralgia and myalgia (*TRI* 327, β 13), anemofrigid cold (*TRI* 500, β 13), diarrhea (*TRI* 427, β 24), internal lesion caused by overexertion (*TRI* 327, β 13), toothache (*TRI* 514, β 3), stroke and paralysis (*TRI* 377, β 6), ulcer disease and sores (*TRI* 528, β 20), and snakebite (*TRI* 400, β 10). The plants of the genus *Delphinium* have a broad-spectrum of bioactivities, such as anti-inflammatory, immunosuppressive, analgesic, anti-tumor, cardiotonic, anti-hypertensive, vasodilative, and other effects. Diterpenoid alkaloids show anti-cancer activity [14]. C_{19}-Diterpenoid alkaloids exhibit cardiac activity [55]. Isoxylitone, an anticonvulsant, modulates voltage-gated, sodium channel inactivation and prevents kindling-induced seizures [56]. *Delphinium* extracts and alkaloids show anti-inflammatory effects against arthritis [57]. Alkaloids show antiparasitic and insecticidal activities [58]. Flavonoids from *Delphinium* show leishmanicidal and trypanocidal activities [59-60]. A *Delphinium* extract shows protective effects against Parkinson's disease through antioxidant activity [61] and a significant effect against morphine-induced tolerance and dependence in mice [62]. The whole plant of *Consolida* is used as an analgesic in the treatment of rheumatic disease and numbness [2]. The seed and the whole plant are used against parasites and insects.

Aconitum is used for various purposes, such as twind-dispelling and damp-drying (*TRI* 5 582, β 64), blood-activating and stasis-removing (*TRI* 999, β 30), interior-warming and cold-dissipating (*TRI* 313, β 4), and the treatment of traumatic injury (*TRI* 4 489, β 59), arthritis (*TRI* 6 488, β 66), neuropathic pain (*TRI* 433, β 19), stroke and paralysis (*TRI* 1 230, β 16), cold and pain of stomach (*TRI* 400, β 4), gastroenteritis (*TRI* 582, β 11), menstrual disorders (*TRI* 320, β 4), and ulcers and sores (*TRI* 2 701, β 52). The anti-cancer activity, cardioactive effects, analgesic activity, anti-inflammatory effects on energy metabolism, antimicrobial,

Table 2 Top eleven ethnopharmacological use of Ranunculaceae plants

Taxon	Heat-clearing and detoxification	Ulcer disease and sore	Anti-microbe and anti-inflammation	Traumatic injury	Wind-dispersing and damp-eliminating	Blood-activating and Stasis-removing	Arthritis	Swell-reducing and detoxification	Dysentery	Pesticide	Antitussive and expectorant
Ranunculoideae											
Ranunculeae											
Ranunculus		+	+				+	+		+	
Batrachium											
Halerpestes					+		+				
Oxygraphis					+						
Anemoneae											
Clematis		+	+	+	+	+	+	+	+		+
Naravelia											
Pulsatilla	+								+		
Anemone	+	+	+	+	+		+	+	+	+	+
Hepatica				+							
Callianthemeae	+		+								
Asteropyreae	+		+								
Caltheae					+		+				
Cimicifugeae											
Actaea	+		+								
Cimicifuga	+			+							
Souliea	+	+									
Eranthis											
Beesia	+			+	+		+				
Helleboreae		+		+		+					
Nigelleae			+							+	+
Delphinieae											
Delphinium		+	+	+	+	+	+			+	+
Consolida					+					+	
Aconitum		+		+	+	+	+	+		+	+
Adonideae											
Adonis		+							+		
Trollius			+			+					
Calathodes					+						
Thalictroideae											
Isopyrum	+		+					+			
Dichocarpum	+	+						+			
Aquilegia		+		+		+					
Semiaquilegia	+	+		+				+			
Urophysa						+					
Paraquilegia						+					
Leptopyrum											
Thalictrum	+	+	+	+					+		+
Coptidoideae	+	+	+						+		
frequency	13	13	12	11	10	8	8	7	6	6	6

Table 3 Top nine bioactivities of Ranunculaceae plants

Taxon	Anticancer/ cytotoxic	Antimicrobial	Antiinflammatory	Immunomodulatory	Analgesic/ sedative	Antihypertensive	Antioxidant	Antiparasitic/ pesticide	Hepatoprotective
Ranunculoideae									
Ranunculeae									
Ranunculus	+	+	+		+	+		+	
Anemoneae									
Clematis	+	+	+		+	+			+
Pulsatilla	+	+	+	+				+	
Anemone	+	+	+	+	+				
Callianthemeae		+	+						
Caltheae			+	+					
Cimicifugeae									
Actaea					+	+			
Cimicifuga	+	+	+	+	+				+
Anemonopsis	+								
Beesia	+			+					
Helleboreae	+	+	+	+	+		+		
Nigelleae	+	+	+	+	+		+		
Delphinieae									
Delphinium	+	+	+	+	+	+	+	+	
Aconitum	+	+	+		+			+	
Adonideae									
Adonis	+								
Trollius		+		+			+	+	
Thalictroideae									
Isopyrum		+							
Aquilegia	+	+		+			+		+
Semiaquilegia	+		+						
Thalictrum	+	+			+	+			
Coptidoideae	+	+	+	+	+	+			
Hydrastidoideae			+						+
Glaucidioideae	+								
Frequency	17	15	14	11	11	6	5	5	4

and insecticidal activities are well-described [15].

Nigelleae

In India, the seeds are used as a carminative and stimulant to ease bowel and indigestion problems, and are given to treat intestinal worms and nerve defects, to reduce flatulence and induce sweating. The dried pods are sniffed to restore a lost sense of smell, and are also used to repel insects. The seeds of *Nigella* are used in diuresis [2]. *Nigella* has anti-inflammatory, analgesic, heat dissipation, antibacterial, and antitumor effects, as well as antitussive and expectorant effects [2]. Thymoquinone attenuates lipid peroxidation and shows a neuroprotective effect [63]. Thymoquinone also inhibits topoisomerase IIα activity [64], induces apoptosis, and displays anticancer activity. The essential oil and oleoresins show *in vitro* antioxidant and antimicrobial activities [18]. *Nigella* is a source of bacterial urease inhibitor [65]. *Nigella* seeds have hypolipidemic effects in menopausal women [66]. The seed oil improves semen quality in infertile men [67].

Increased 5-HT levels following repeated administration of *Nigella* oil produce antidepressant effects in rats [68]. The *Nigella* extract prevents scopolamine-induced spatial memory deficits and decreases the acetylcholinesterase activity as well as oxidative stress of brain tissues in rats [69-70]. The seed extracts show immunomodulatory effects on human peripheral blood mononuclear cells [19].

Helleboreae

The genus *Helleborus* is used for the treatment of traumatic injury (*TRI* 500, *β* 100), urinary tract infection (*TRI* 500, *β* 100), ulcers and sores (*TRI* 500, *β* 100), and internal lesions caused by overexertion (*TRI* 320, *β* 100). Bufadienolides

exhibit potent cytotoxic activities against cancer cells [71] and also have antibacterial activity [72]. Polyphenolic extracts exhibit inhibitory activity against urease, and low inhibition against α-chymotrypsi, and could be used in ulcer treatment [73]. A fraction from *Helleborus* modulates HMGB1 cytokines and attenuates septic shock in mice [74]. *Helleborus* shows antioxidant and antiproliferative activities [75], as well as anti-inflammatory and antinociceptive activities [76]. MCS-18, a macrocyclic carbon suboxide $(C_3O_2)_n$ derivative, exerts immunosuppressive [77], immunomodulatory [78], and analgesic activities [79].

Cimicifugeae

Cimicifuga is used in various settings such as wind-heat dispersing (*TRI* 1 350, *β* 57), heat-clearing and detoxification (*TRI* 1370, *β* 71), benefiting Qi and raising Yang (*TRI* 1350, *β* 57), swelling and pain in throat (*TRI* 776, *β* 85), variola and exanthema (*TRI* 400, *β* 71), archoptosis (*TRI* 1112, *β* 85), and uterine prolapse (*TRI* 704, *β* 57). *Actaea* is used in wind-heat dispersing (*TRI* 300, *β* 50), and heat-clearing and detoxification (*TRI* 300, *β* 50). *Souliea* is used in heat-clearing and detoxification (*TRI* 320, *β* 100), and ulcers and sores (*TRI* 500, *β* 100). *Beesia* is used in wind-dispelling and damp-drying (*TRI* 400, *β* 100) and arthritis (*TRI* 400, *β* 100). The whole plant of *Eranthis* is used in diuresis and for urinary stones [2].

The Cimicifugeae has anti-inflammatory, antipyretic, analgesic, antivirus, detoxification, and estrogen-like activities. The anticancer activity, effects on menopausal symptoms and cardiovascular system, osteoprotective effects, and immunosuppressive activity are well-described [22]. The cycloartane triterpenoids show anticancer, immunomodulatory, and hepatoprotective activities, as well as effects on the cardiovascular system [80].

Caltheae

Caltha is used in the treatment of arthritis (*TRI* 400, *β* 100). The polysaccharide fraction shows anti-arthritis and immunomodulatory activities [81].

Asteropyreae

Asteropyrum species are used by minority ethnic groups of southwest China in heat-clearing and detoxification, damp-drying and diuresis, and the treatment of dysentery, jaundice, ulcers and sores, and traumatic injury [25].

Callianthemeae

Callianthemum has detoxifying and anti-inflammatory activity, and is used in various diseases, including children's pneumonia and drug-fire eyesight [26].

Anemoneae

Anemone is used in heat-clearing and detoxification (*TRI* 424, *β* 30), wind-dispersing and damp-eliminating (*TRI* 476, *β* 35), warming and orifice-opening (*TRI* 700, *β* 15), pesticide (*TRI* 400, *β* 30), dysentery (*TRI* 1 051, *β* 46), malaria (*TRI* 356, *β* 30), tinea (*TRI* 445, *β* 46), ulcers and sores (*TRI* 1 932, *β* 84), arthritis (*TRI* 896, *β* 76), traumatic injury (*TRI* 930, *β* 53), pharyngolaryngitis (*TRI* 327, *β* 7), parasitic disease (*TRI* 424, *β* 30), and hepatitis (*TRI* 445, *β* 7). A broad spectrum of pharmacological activities, including antitumor, antimicrobial, anti-inflammatory, sedative and analgesic activities, and anti-convulsant and anti-histamine effects have been observed [27]. Triterpenoid saponins having anticancer, antibacterial and anti-arthritis properties were isolated. The genus *Pulsatilla* is used in heat-clearing and blood-cooling (*TRI* 1 125, *β* 50), heat-clearing and detoxification (*TRI* 845, *β* 50), dysentery (*TRI* 1 570, *β* 100), hemorrhoid (*TRI* 405, *β* 50) and nasal hemorrhage (*TRI* 337, *β* 33). *Pulsatilla* has anti-tumor, antibacterial, anti-inflammatory, immune enhancing, and anti-trichomonal effects [2].

Clematis is used in wind-dispelling and damp-eliminating (*TRI* 3 788, *β* 52), blood-activating and stasis-dispelling (*TRI* 1 067, *β* 33), damp-filtering and diuresis (*TRI* 1 824, *β* 27), heat-clearing and damp-drying (*TRI* 578, *β* 20), swell-reducing and detoxification (*TRI* 678, *β* 29), and the treatment of arthritis (*TRI* 4 848, *β* 58), traumatic injury (*TRI* 1 778, *β* 37), urinary tract infection (*TRI* 1 972, *β* 45), bones stuck in the throat (*TRI* 1 536, *β* 12), agalactia (*TRI* 1 869, *β* 18), abnormal menstruation (*TRI* 1 393, *β* 33), nephritis and edema (*TRI* 845, *β* 25), dysentery (*TRI* 632, *β* 18), ulcers and sores (*TRI* 1 000, *β* 37), stomatitis (*TRI* 612, *β* 14), toothache caused by wind-fire (*TRI* 511, *β* 14), and snakebite (*TRI* 352, *β* 18). *Clematis* has anticancer, antibacterial, anti-inflammatory, analgesic, sedative and plant hormone-like effects [28], and is used in myocardial ischemia and chronic cholecystitis. Diuretic, antiarthritis, hepatoprotective, hypotensive, and HIV-1 protease inhibitor activities are also revealed [29]. The whole plant of *Naravelia* is used in Qi-moving and pain-relieving [2]. The root and stem of *Hepatica* are used in traumatic injury, internal lesions caused by overexertion, arthralgia, and myalgia [2].

Ranunculeae

Ranunculus is used as a parasiticide (*TRI* 785, *β* 21), for swell-reducing and detoxification (*TRI* 326, *β* 50), malaria (*TRI* 3 291, *β* 57), scrofula (*TRI* 1 525, *β* 64), arthritis (*TRI* 568, *β* 42), asthma (*TRI* 785, *β* 21), jaundice (*TRI* 589, *β* 28), and ulcers and sores (*TRI* 944, *β* 50). The genus *Ranunculus* has anticancer, anti-inflammatory, anti-oxidant, analgesic, anti-microbial, anti-parasitic, and cardiovascular effects. *Halerpestes* is used in wind-dispelling and damp-eliminating (*TRI* 400, *β* 100), and for arthritis (*TRI* 400, *β* 100) and edema (*TRI* 400, *β* 100). The whole plant of *Oxygraphis* is used in wind-dispelling and cold-expelling, orifice-opening, and collateral-dredging [2].

Thalictroideae

The genus *Thalictrum* is used in heat-clearing and detoxification (*TRI* 1 731, *β* 54), heat-clearing and damp-drying (*TRI* 682, *β* 33), heat-clearing and blood-cooling (*TRI* 300, *β* 6), and the treatment of dysentery (*TRI* 2 807, *β* 69), gastroenteritis (*TRI* 1 340, *β* 48), red eye with swelling and pain (*TRI* 2 625, *β* 63), hepatitis (*TRI* 306, *β* 12), diarrhea (*TRI* 327, *β* 12), jaundice (*TRI* 804, *β* 27), ulcers and sores (*TRI* 1 225, *β* 54), stomatitis (*TRI* 776, *β* 30), abdominal pain (*TRI* 583, *β* 33), vomiting (*TRI* 465, *β* 30), infantile

convulsions (*TRI* 492, *β* 12), and indigestion in children (*TRI* 320, *β* 6). *Thalictrum* species have anticancer, antibacterial, anti-hypertensive, anti-arrhythmia, spasmolytic, analgesic, and sedative effects [5, 8]. The saponins have immunomodulatory activity. The genus *Leptopyrum* is used in gastrointestinal diseases, and *Paraquilegia* is used in blood-activating and stasis-resolving, as well as for traumatic injury. In Tibetan medicine, it is used for treating uterine hemorrhage and after giving birth to a still-born [8].

Semiaquilegia species are used in heat-clearing and detoxification (*TRI* 1 164, *β* 100), damp-filtering and diuresis (*TRI* 476, *β* 50), and the treatment of ulcers and sores (*TRI* 1 473, *β* 100), scrofula (*TRI* 1 473, *β* 100), traumatic injury (*TRI* 550, *β* 100), snakebite (*TRI* 805, *β* 50), and urinary stones (*TRI* 305, *β* 50). *Semiaquilegia* has antitumor and anti-inflammatory activities [82-83].

The genus *Aquilegia* is used in the treatment of menstrual disorders (*TRI* 600, *β* 60) and uterine hemorrhage (*TRI* 400, *β* 40). *Aquilegia* flavonoids have antioxidant, antimicrobial, and hepatoprotective effects [84]. *Aquilegia* is also used in epilepsy and as a hypnotic. The saponins show immunosuppressive activity [84], and the alkaloids have cytotoxic activity [36]. The root of *Urophysa* is used in blood-activating and stasis-removing [2]. *Isopyrum* has antibacterial activity and is used in reducing swelling,resolving mass, and detoxification. *Dichocarpum* is used in swell-reducing and detoxification (*TRI* 300, *β* 20), for ulcers and sores (*TRI* 300, *β* 20) and dyspepsia in children (*TRI* 300, *β* 20).

Coptidoideae

Coptis is used in heat-clearing and detoxification (*TRI* 847, *β* 60), heat-eliminating and fire-purging (*TRI* 588, *β* 60), heat-clearing and damp-drying (*TRI* 588, *β* 20), anti-microbe and anti-inflammation (*TRI* 300, *β* 20), and the treatment of dysentery (*TRI* 1 032, *β* 100), gastroenteritis (*TRI* 712, *β* 80), red eye with swelling and pain (*TRI* 758, *β* 100), stomatitis (*TRI* 526, *β* 100), and ulcer disease and sore (*TRI* 889, *β* 100). *Coptis* has antibacterial, antiviral, antipyretic, anticancer, immunomodulatory, spasmolytic, anti-diarrhea, anti-gastric ulcer, hypoglycemic, anti-inflammatory, anti-hypertensive, anti-platelet aggregation, anti-arteriosclerosis, and anti-arrhythmia activities [5].

Hydrastidoideae and Glaucidioideae

Hydrastis species have anti-catarrhal, anti-inflammatory, and antiseptic activities, and are used as an astringent, bitter tonic, laxative, anti-diabetic, and muscular stimulant. The astringent effect is on the mucous membranes of the upper respiratory tract, the gastrointestinal tract, the bladder, the rectum, and the skin. *Hydrastis* species stimulates the appetite and bile secretion, and aids digestion. Glaupalol-related coumarins from *Glaucidium* show antimitotic activity [43].

Discussion

The relationship between the chemical composition of the Ranunculaceae and systematics

There are about 60 genera and 2 200 species in the family Ranunculaceae, leading to a complex and diverse chemical composition. The most prominent alkaloids are of the benzylisoquinoline, bisbenzylisoquinoline, apophinoid (*e.g*., magnoflorine), and protoberberine types. Triterpenoid saponins, lactones (*e.g*., ranunculin), cyanogenic glycosides, and flavonoids are also commonly found in some genera (Table 1). Flavonoids are present in most of the genera. Other compounds are distributed selectively, reflecting the phylogenetic relationships between taxonomic groups.

It is believed that ranunculin and magnoflorine appear alternately [2]. However, they are now found to co-exist in *Clematis*, *Caltha* and *Helleborus*. Both *α*- and *β*-magnoflorines are isolated from the aerial parts of *Clematis parviloba* [86]. Magnoflorine is also isolated from *Clematis recta* and *Helleborus viridis* [87]. *Caltha* species contain the irritant glycoside ranunculin [88]. These results suggest that it is reasonable to put these genera into the same subfamily, Ranunculoideae.

The chemical profiles of *Cimicifuga*, *Actaea*, *Souliea*, and *Beesia* are similar. Triterpenoid saponins, especially those of the cycloartane type, are abundant in these genera. Magnoflorine and ranunculin are not found, which is distinct from other genera of Ranunculoideae plants, therefore justifying the treatment of the Cimicifugeae as a separate tribe.

None or very few benzylisoquinoline alkaloids are found in the Ranunculeae, Anemoneae, and Callianthemeae (Table 1). The Ranunculeae and Anemoneae are rich in saponins, especially the pentacyclic triterpenoid derivatives, embodying the close relationship between these tribes.

Cyanogenic glycosides are the outstanding feature of the subfamily Thalictroideae. Thalictroideae and Coptidoideae are rich in alkaloids. Benzylisoquinoline and bisbenzylisoquinoline alkaloids are abundant in the Thalictroideae, while protoberberine alkaloids are copious in the Coptidoideae. *Thalictrum*, *Paraquilegiai*, and *Aquilegia* species contain triterpenoid saponins, *e.g*., cycloartane saponins, indicating the close relationship between the Thalictroideae and Ranunuloideae. The sterile stamens of *Aquilegia* and *Semiaquilegia* species are very similar morphologically, and these two genera also have very similar flavonoid *C*-glycosides, suggesting their close phylogenetic relationship.

Benzylisoquinoline alkaloids are of the monophyletic origin [89-90]. Ranunculin is only abundant in the tribes Ranunculeae, Anemoneae, and Helleboreae (Table 1) [2]. Diterpene alkaloids are characteristic of the tribe Delphinieae [2], although a few types and a low level of diterpene alkaloids have been detected in *Nigella* and *Thalictrum* species [15, 17]. Ferulic and sinapic acids are abundant in *Trollius* and *Adonis* species, and are present in the genus *Thalictrum* in small amounts [91]. These chemotaxonomic markers, along with the molecular markers, morphology, and cytology data, have shown their utility in the comprehensive analysis of the phylogeny of the Ranunculales [7]. Similarly, volatile

compounds, such as fatty acid derivatives, benzenoids, phenylpropanoids, nitrogen-containing compounds and terpenes, are promising chemotaxonomic markers [11]. In the future, based on the accumulated metabolomic data of the respective genus, triterpenoid saponins, cardiac glycosides, and cyanogenic glycosides could be useful in the phylogenetic analysis of the Ranunculaceae.

The relationship between chemical composition and therapeutic effects of the Ranunculaceae

Many Ranunculaceae plants are traditionally used in heat-clearing and detoxification (Table 2), the treatment of ulcers and sores, as anti-microbials, and as anti-inflammatories, but the effective ingredients may vary. For example, ranunculin and protoanemonin lactone, triterpenoid saponins, and benzylioquinoline and protoberberine alkaloids have antibacterial activity.

The Cimicifugeae are used in wind-dispersing and detoxification, as well as promoting eruption. The use of Cimicifugeae in benefiting Qi and raising Yang, archoptosis, and terine prolapse is unique among Ranunculaceae plants, and is distinct from the ethnopharmacological use of the closely related tribe Helleboreae. The estrogen-like effects of *Cimicifuga* (*Actaea*) *racemosa* are exceptional, as no plants of other tribes/sub-families show such activities.

Many saponins have anticancer/cytotoxic activity (Table 3). Most saponin-containing plants, *e.g*., those of the Ranunculeae, Anemoneae, Cimicifugeae, Nigelleae, and Thalictroideae, are used in swelling-reduction and pain-relief, and for ulcers and sores. These plants are a gold mine for searching for drug-like anticancer compounds.

The tribe Delphinieae is rich in unique diterpenoid alkaloids, which have a broad spectrum of pharmacological effects (Table 3), not limited to analgesic and anti-arthritic activities. Small amounts of diterpenoid alkaloids are found in the genera *Thalictrum*, *Nigella*, and *Clematis*, which may contribute to the versatile therapeutic use of these genera, but their exact modes of action await further study.

The Thalictroideae and Coptidoideae are rich in benzylisoquinoline alkaloids, which make them a rich resource for antibacterial, antiviral, and anticancer compounds. In addition, the Thalictroideae, with bisbenzylisoquinoline alkaloids, is used in heat-clearing and blood-cooling. They may be promising lead compounds against hypertension and other cardiovascular diseases.

The common traditional perception of Ranunculaceae plants therefore requires revision, as they possess more general properties and/or novel bioactivities than previously considered. In a broader context, ethnopharmacologic indications for all herbal remedies should be revisited in light of the explosion in understanding of the modes of action of small molecule effectors, of which the metabolites of the Ranunculaceae are only representative examples.

Conclusions

Biodiversity, as a result of its compositional chemical diversity, has served as one of the richest sources of bioprospecting, resulting in the discovery of some of the most important clinical drugs [5, 15]. Ranunculaceae plants have a huge reservoir of chemical constituents, which are not distributed randomly, thus having taxonomic implications. Alkaloids, saponins, and ranunculin are the main chemical features and could be useful in chemotaxonomy. The new chemical profile data, based on the more sensitive analytical technology, argue that the distribution of ranunculin and magnoflorine are not mutually exclusive. We agree with the system proposed by Wang *et al*. [7] which divides the Ranunculaceae into five sub-families, and the sub-family Ranunculeae into ten tribes. More species could be discovered in biodiversity hotspots, and there is a lack of chemical data in many Ranunculaceae genera, *e.g*., *Dichocarpum*, *Paraquilegia*, *Urophysa*, *Hepatica*, *Naravelia*, *Oxygraphis*, and *Halerpestes*, implying that the chemical and biological space for exploration is still wide open. Pharmacophylogeny, as a fascinating approach for mining chemodiversity from biodiversity, could be further developed and used in the future to accelerate the pace of drug discovery and development.

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