Mining Therapeutic Efficacy from Treasure Chest of Biodiversity and Chemodiversity: Pharmacophylogeny of Ranunculaceae Medicinal Plants

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ABSTRACT Ranunculales, comprising of 7 families that are rich in medicinal species frequently utilized by traditional medicine and ethnomedicine, represents a treasure chest of biodiversity and chemodiversity. The phylogenetically related species often have similar chemical profile, which makes them often possess similar therapeutic spectrum. This has been validated by both ethnomedicinal experiences and pharmacological investigations. This paper summarizes molecular phylogeny, chemical constituents, and therapeutic applications of Ranunculales, i.e., a pharmacophylogeny study of this representative medicinal order. The phytochemistry/metabolome, ethnomedicine and bioactivity/pharmacology data are incorporated within the phylogenetic framework of Ranunculales. The most studied compounds of this order include benzylisoquinoline alkaloid, flavonoid, terpenoid, saponin and lignan, etc. Bisbenzylisoquinoline alkaloids are especially abundant in Berberidaceae and Menispermaceae. The most frequent ethnomedicinal uses are arthritis, heat-clearing and detoxification, carbuncle-abscess and sore-toxin. The most studied bioactivities are anticancer/cytotoxic, antimicrobial, and anti-inflammatory activities, etc. The pharmacophylogeny analysis, integrated with both traditional and modern medicinal uses, agrees with the molecular phylogeny based on chloroplast and nuclear DNA sequences, in which Ranunculales is divided into Ranunculaceae, Berberidaceae, Menispermaceae, Lardizabalaceae, Circaeasteraceae, Papaveraceae, and Eupteleaceae families. Chemical constituents and therapeutic efficacy of each taxonomic group are reviewed and the underlying connection between phylogeny, chemodiversity and clinicial uses is revealed, which facilitate the conservation and sustainable utilization of Ranunculales pharmaceutical resources, as well as developing novel plant-based pharmacotherapy.

KEYWORDS Ranunculales, chemical constituent, bioactivity, diversity, pharmacophylogeny

Ranunculales is an order of angiosperms, containing 7 families, i.e., Ranunculaceae (at least 50 genera, >2,000 species), Berberidaceae (17 genera, 650 species), Menispermaceae (65 genera, >350 species), Lardizabalaceae (9 genera, 50 species), Circaeasteraceae (2 species), Papaveraceae (38 genera, >700 species), and Eupteleaceae (2 species, Appendix 1). Ranunculales belongs to the basal eudicots, in which it is the most basal clade; its widely known members include poppies, barberries, monseed, akebia and buttercups. The chemodiversity of Ranunculaceae, the flagship family of Ranunculales, and their correlation with the biodiversity and pharmacotherapy, have been systematically reviewed by us in the context of pharmacophylogeny. In recent years, the research enthusiasm for the other 4 major Ranunculales families has been growing rapidly, as plants of these families also meet human needs for food, flavours, fragrances, and not the least, medicines during long history. Ranunculales plants form the basis of sophisticated traditional medicine systems, e.g., Chinese medicine (CM) and Ayurveda medical system. These systems of medicine give rise to some important Ranunculales-based drugs still in use today. According to incomplete statistics, 573 Ranunculaceae and 308 Berberidaceae species were in medicinally hot nodes; the therapeutic efficacy of 2 families differed substantially. For instance, up to 361 Chinese Ranunculaceae species are used against musculoskeletal diseases, followed by 321 against hepatic diseases, 242 against circulatory diseases, and 210 against skin diseases, etc.; in contrast, as many as 251 Chinese Berberidaceae species are used against eyesight diseases, followed by 249 against hepatic diseases, 242 against digestive diseases and 90 against oral diseases, etc. Nevertheless, except Ranunculaceae, the
phytochemistry/metabolome, ethnomedicine and bioactivity/pharmacology data of other Ranunculales families have not been incorporated within the phylogenetic framework of Ranunculales and related basal eudicots. Ethnobotany and ethnomedcine/metabolome lend support to the nowadays search for new molecules of different sources and classes. The flora of the tropics, Southwest China and Himalayas is fantastic and contains the most diverse Ranunculales taxa. The biodiversity of these hotspot regions plays a significant role in providing new drug leads for the innovative and improved clinical treatments, although the sovereignty and property rights should be addressed along with the convention for biological diversity.

The present study highlights the recent progress of Berberidaceae, Menispermaceae, Lardizabalaceae and Papaveraceae, provides an overview of the classes of molecules present in Ranunculales plants and gives examples of the types of molecules and secondary metabolites that lead to the development of pharmacologically active extracts/preparations. We assume that the phylogenetic framework is helpful to recognize the distribution law of compounds and efficacies, and to analyze the cryptic link between bioactive compounds and clinical efficacies. Only by mastering the distribution regularity of compound bioactivity/toxicity can Ranunculales plant products be used to develop functional foods. This study simultaneously focuses on the front, middle and back of pharmacophylogeny, trying to find a trade-off. The pharmacological validation of Ranunculales extracts/preparations is indispensable, and safety, efficacy and quality of phyto-medications should always be emphasized.

Systematics and Evolution of Ranunculales

Around 75% of all angiosperm species belong to the eudicot clade, which is strongly supported by molecular data and the morphology of single synapomorphy-tribiparturate pollens. Ranunculales is a basal clade of eudicots, which is sister to all other eudicots (Appendix 2), e.g., Saxifragales, Caryophyllales, rosids, and asterids. Despite the progress in resolving angiosperm phylogenetic relationships, the branching order among basal eudicots remains an issue; while fossils should be integrated with extant taxa into a comprehensive tree of angiosperm phylogeny, the chemotaxonomic evidence is also essential. Many phylogenetic studies suggest that Eupteleaceae, an East Asian family of 2 tree species with perianthless flowers, is sister to all remaining Ranunculales, with Papaveraceae branching next (Appendix 1B). Lardizabalaceae and Circaeeastereae cluster together, which are basal to the clade containing Menispermoideae, Berberidaceae and Ranunculaceae; Berberidaceae and Ranunculaceae are closer to each other than to other families.

All the lineages of basal eudicots emerged during the latest part of the Early Cretaceous. Among early-diverging eudicots, the age of Ranunculales was around 120 my, older than Proteales (119 my), Sabiales (118 my), Buxales (117 my) and Trochodendrales (116 my). Recently the complete plastome sequences of many early-diverging eudicot taxa, e.g., Epimedium sagittatum (Berberidaceae), Euptelea pleio sperma (Eupteleaceae), Akebia trifoliata (Lardizabalaceae), Stephania japonica (Menispermoideae) and Papaver somniferum (Papaveraceae), are used to elucidate the evolution of plastome structure and the phylogenetic correlation. The maximum likelihood phylogenetic analysis of a 79-gen, 97-taxon data set that included all available early-diverging eudicots largely agreed with previous estimates of phylogenetic relationships (Appendix 2).

Menispermoideae consists of 2 subfamilies: Tinosporoideae and Menispermoideae (Appendix 1A). Within Tinosporoideae, tribe Coscineae is basal; within Menispermoideae, tribe Menispermeae is basal. Tinosporoideae taxa usually have apical style scars, bilateral curvature, subhemispherical condyles, and foliaceous cotyledons with divaricate or imbricate orientation. Menispermoideae taxa generally have basal or subbasal style scars, dorsoventral curvature, bilaterally and/or dorsoventrally compressed condyles, and subterete or fleshy cotyledons oriented dorsoventrally or laterally. Most of the genera with more pharmaceutical research belong to Menispermoideae. In Papaveraceae (Appendix 1B), the subfamily Fumarioideae (20 genera, 593 species) is characterized by flowers that are either disymmetric (i.e. 2 perpendicular planes of bilateral symmetry) or zygomorphic (i.e. 1 plane of bilateral symmetry). In contrast, the subfamily Papaveroideae (23 genera, 230 species) has actinomorphic flowers (i.e. more than 2 planes of symmetry). Six plastid markers and 1 nuclear marker were used to infer the phylogenetic relationship of Pteridophyllum, 73 species of Fumarioideae and 11 species of Papaveroideae. Pteridophyllum is not nested in Fumarioideae. Fumarioideae are monophyletic and Hypecoum (18 species) is the sister group of the remaining genera. Relationships within the core Fumarioideae are well resolved and supported (Appendix 1B). Dactylicapnos and zygomorphic genera, e.g., Fumaria and Corydalis, form a well-supported clade nested among disymmetric taxa. As compared with Fumarioideae, more Papaveroideae taxa are medicinally studied.

Chemical Composition of Berberidaceae

Phylogenetically and phytochemically, Berberidaceae is closer to Ranunculales than to other Ranunculales families (Appendix 1). It is divided into 3 subfamilies: Nandinaoidae, Berberidoideae, and Podophyloideae. Nandinaoidae is characterized by a rich spectrum of benzylisoquinoline alkaloids (BIAs). The cyanogenic nandinin, biflavonoid amentoflavone and benzaldehyde-4-O-glucoside in this family indicates its relatively distant relation with other subfamilies. Berberidoideae contains mainly BIAs, particularly a higher content of bisbenzylisoquinoline (BBI) represented by berbamine and oxyacanthine. Podophyloideae is divided into 2 tribes. The tribe Podophyllum contains extensively various podophyllotoxin lignans, while the tribe Epimediaceae has diverse constituents such as icariin flavonoids.
Gymnospermium, Leontice, Caulophyllum and Bongardia contain β-amyrin triterpenoids and quinolizidine alkaloids.

**Nandinoideae Subfamily**

Phylogenetically, the genus Nandina is basal to Caulophyllum and Gymnospermium (Appendix 1); it is rich in various BIAs, e.g., berberine, palmatine, jatrorrhizine, coptisine, magnoflorine, domesticine, nandinine and protopine. Steroidal alkaloids, e.g., nandsterine, are also found in the fruit (Appendix 3). The piperidines and N-containing xanthone derivative were isolated from the roots of Caulophyllum robustum.

The aporphine alkaloids, magnoflorine, corydaldine, noroxyhydrastinine, 9-epi-acortatarin A, vomifoliol, and the number of type I–Ⅺ were isolated from the fruits of Nandina domestica. Phenolic 1-benzyl-N-methyltetrahydroisookinolines are present in cell cultures of Corydalis, Macleaya, and Nandina. The benzyltetrahydroisookinolyl alkaloid higenamine exists in Aconitum. The cyanogenic compound nandinin, β-amyrin triterpenoids and quinolizidine alkaloids. Aporphine BIAs were found in at least 6 Berberidaceae genera (Appendix 1), e.g., Epimedium, Podophyllum, Mahonia, Berberis, Caulophyllum and Nandina.

**Chemical Composition of Menispermaceae**

Menispermaceae is a medium-sized family of 70 genera and 420 extant species, mostly climbing plants. It has various medicinal properties, which are used in CM and the Ayurvedic system of medicine. Menispermaceae plants are rich in alkaloids, especially BBI type (Appendix 5). The number of type Ⅴ is the most, followed by Ⅰ, Ⅶ, Ⅷ and Ⅺ (Appendices 6 and 4). BBI alkaloids, morphine alkaloids, aporphine alkaloid, syringaresinol (lignan) and aristolochic acid Ⅰ (organic acid) could be marker compounds of this family.

**Stephania**

Phylogenetically, Stephania is closer to Cyclaea and Cissampelos than to Cocculus, Sinomenium and Menispermum in subfamily Menispermoideae (Appendix 1). Interestingly, Stephania and Cyclaea/Cissampelos share 5 types of BBIs (Appendix 4), while it and Cocculus share 4 BBIs; these 4 genera share type Ⅵ, Ⅶ and Ⅷ BBIs. The alkaloids of S. rotunda were classified into 9 structural families: phenanthrene, quinoline, isouquinoline, benzyltetrahydroisookinoline, protoberberine, tetrahydroprotoberberine, aporphine, oxaapomorphine, and morphinan derivative, etc. Alkaloids of S. tetrandra were classified into 6 structural classes, including monobenzyltetrahydroisookinoline, BBI, aporphine, prototberberine and tetrahydroprotoberberine, etc. An aporphine glycoside angkorwatine and 8 alkaloids: oblongine, stepharine, asimilobine-β-d-glucopyranoside, nandinamegastigmanes, β-amyrin triterpenoids and quinolizidine alkaloids. Aporphine BIAs were found in at least 6 Berberidaceae genera (Appendix 1), e.g., Epimedium, Podophyllum, Mahonia, Berberis, Caulophyllum and Nandina.
isolated from the stems of *Cyclea barbata*.\(^{[43]}\) Cissampentine A, an enantiomer of cissampentin, and cycleatjehenine-type BBI alkaloids, cissampentine B–D, were isolated from the roots of *C. tonkinensis*.\(^{[44]}\) Curine-type BBI alkaloids are found in the roots of *C. watii*.\(^{[45]}\) Racemosidine A, isolated from the roots of *C. racemosa*, is a BBI alkaloid that has a diphenyl ether bridges at C-11/C-7’ and C-8/C-12’ and a benzyl-phenyl ether bridge at C-7’/C-11.*\(^{[46]}\)* Phytosterols and alkaloids are major phytocenstituents in petroleum ether extract of *C. peltata* leaf.\(^{[47]}\)

The ethanolic extract of *C. peltata* leaf showed the presence of alkaloids, flavonoids, tannins, diterpenes and saponins.

Ten skeletal types were identified in alkaloids of *Cissampelos*: benzylisoquinoline, BBI, aporphine, morphinidine, tetrahydroprotoberberine, tropolisoquinoline, azafuoranthenone, pro-aporphine, stephaoxocan, and pyrrolic nuclein-derived alkaloid, etc.\(^{[48]}\) The phylogenetically close *Cissampelos* and *Cyclea* share type V, VI, VII, XX, XI, XII and XXI BBIs (Appendix 4), and their BBI profiles are closer to *Coccus* than to *Menispermum*, which is in accordance with *Cissampelos* and *Cyclea* sharing type bridges at C-7/C-11.\(^{[49]}\)

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Sinomenium and *Menispermum* might lie in BBI, possibly due to the lack of BBI study on the former. Numerous BBIs, mainly tail-to-tail with one diphenyl ether linkage, are identified from the latter. N-oxides of dauricine-type BBIs and rare tail-to-tail quaternary alkaloids were identified from the rhizome of *Menispermum dauricum*.\(^{[50]}\) N-methylcorydaline, thalifoline, stepholdine, acutumine, daurisoline, acutumidine, dauricicoline, fumaguricine, 6-O-demethylneporphine, fumagudine, dauricoside, eleutheroside D, aristolactone, aristotriterpenate I and aristolochic acid were identified from *M. dauricum*.\(^{[51]}\)

**Tinospora**

Alkaloids (including protoberberine, aporphine BBIs, sesquiterpenoids, diterpenoids (mainly clerodane type), phenolics, steroids, aliphatic compounds and polysaccharides were found in *Tinospora cordifolia*.\(^{[52]}\) In *T. sinensis*, the amide alkaloids were the most,\(^{[53]}\) followed by organic amine alkaloids, imidazole alkaloids, guanidine alkaloids, purine alkaloids, pyrroline alkaloids, piperidine alkaloids, indole alkaloids, and pyrimidine, etc. Crispene A, B, C and D, clerodane type furanoid diterpenes, and a furanoid diterpene glucoside borapetoside E were isolated from the stem of *Tinospora crispa*.\(^{[54]}\) Clerodane type diterpenoids were also isolated from tuberous roots of *T. sagittata*.\(^{[55]}\)

**Other Genera**

Alkaloids reticuline, asimilobine, acutumine, dihydroxyprotoberberine, and stepholdine were isolated from the vine stems of *Diploclisia affinis*.\(^{[56]}\) Ecdysteroid, (2-nitro ethyl) phenyl and cyanophenyl glycosides, steroidal and triterpenoid saponins were found in the fruits of *D. glaucescens*.\(^{[57]}\) Ecdysteroid and oleanane glycosides are isolated from the leaves of *D. glaucescens*.\(^{[58]}\)

A β-caroline alkaloid sacleuximine A, together with palmatine, isotetrandrine (BBI), trans-N-furulyloyramine, trans-N-caffeoylramine, yangambin, syringaresinol, sesamin, (+)-epi-querctol, 4-hydroxybenzaldehyde, β-sitosterol, quercetin-3-O-rutinoside and myricetin 3-O-β-glucose (1→6) α-ribose, were isolated from *Trilicisia sacleuxii* aerial parts.\(^{[59]}\) This genus, unlike *Diploclisia* and *Sinomenium*, could be an excellent resource of BBIs.\(^{[60]}\)

**Arcangelisia and Fibraurea**

*Arcangelisia* and *Fibraurea* belong to the subfamily Tinosporoideae (Appendix 1). Protoberberine BBIs are isolated from *Arcangelisia gusamun* and *A. flava*.\(^{[61]}\) Fibaruretin B (2 β,3 α -dihydroxy-2,3,7,8 α -tetrohydro-penicilanic acid lactone) was isolated from the roots of *A. flava*.\(^{[62]}\) Furanoditerpenes were found in *A. flava*.\(^{[63]}\)

Palmatine, fibreicine, berberines, tetrahydroberberines and aporphine derivatives are found in *Fibraurea recisa*.\(^{[64]}\) Furanoditerpenoids, e.g., epi-8-hydroxycolobin, fibaruretin B, C, E, and F, were isolated from the stems of *F. tinctoria*.\(^{[65]}\) Twenty-five protoberberine BBIs were present in both *Tinospora Radix* and *Fibraurea Caulis*,\(^{[66]}\) while 5 compounds were detected only
in the former; the contents of 4 alkaloids in Tinosporae Radix were much higher than those in its adulterant, Fibrulaire Caulis.

Chemical Composition of Lardizabalaceae and Circaeasteraceae

Oleanane type triterpenoid saponins, as a characteristic chemical component of Lardizabalaceae, have certain chemotaxonomic significance, which not only suggest the phylogenetic relationship between Lardizabalaceae and Ranunculaceae, but also the oxidation level of aglycone skeleton is related to the phylogeny of various genera in the family.

Sargentodoxa

Over 110 chemical constituents have been identified from the stem of S. cuneata, the only species of Sargentodoxa, including phenolic acids, phenolic glycosides, lignans, flavones, triterpenoids and others. Sargentodoxa is rich in tannins, which are lacking in other genera of Lardizabalaceae. 3,4-Dihydroxyphenylethyl alcohol glycoside, salidroside, tannins, which are lacking in other genera of Lardizabalaceae. Triterpenoid saponins are dominant specialized metabolites of Akebia and Sargentodoxa Caulis is required by the quality standard of the B, were isolated from the stems of Holboellia coriacea.

Akebia

Akebiae Caulis refers to a group of herbal medicines with different phylogenetic positions. A. Caulis is derived from 8 species of families Lardizabalaceae and Ranunculaceae. In the Chinese Pharmacopoeia, it has been separated into 2 categories: A. Caulis and Clematidis Armandii Caulis. A. Caulis is one of the newest raw materials officially introduced into the Chinese Pharmacopoeia from CM; the marker constituents salidroside, chlorogenic acid and 3, 4-dihydroxy-phenylethyl-β-D-glucopyranoside in Sargentodoxa Caulis is required by the quality standard of the crude material recorded in the Chinese Pharmacopoeia.

Chemical Composition of Papaveraceae and Eupteleaceae

Papaveraceae has at least 38 genera, with more than 700 species. Phylogenetically, Papaveraceae is basal to other Ranunculales families except Eupteleaceae (Appendixes 1 and 2). Its chemical profile is distinct as compared with other Ranunculales families.

Stauntonia

Triterpenoid saponins (e.g., oleanane, nor-oleanane and lupane type saponins), flavonoids, lignanoids, and phenylethanoid glycosides were identified in S. brachyanthera, S. chinensis, and S. obovatifoliola. Their structural types are much similar to those of A. quinata, i.e., either penta-saccharidic or hexa-saccharidic bidesmoside triterpenoid glycosides. Calceolarioside B is abundant in both Akebia and Stauntonia. S. brachyanthera could be a succedaneum of Akebia Caulis, which undergoes the supply crisis in recent years. Phenolic acids, flavonoids, organic acids, and calceolariosides were isolated from S. hexaphylla.

Circaeasteraceae

The medicinal compounds of Circaeaster agrestis and Kingdonia uniflora have not been reported.

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Fumarioideae: Corydalis, Fumaria and Hypecoum

Phylogenetically, Corydalis is closer to Fumaria than to Hypecoum (Appendix 5). Among identified Corydalis alkaloids, the quaternary isoquinoline type is the most, followed by benzophenanthridine type, phthalide isoquinoline, simple isoquinoline, aporphine type, lignin amide and protopine type, etc. Isoquinoline alkaloids are the major bioactive ingredients of Corydalis. Eight types of alkaloids were found in Chinese Corydalis: protopine; protoberberine; phthalide; benzophenanthridine; aporphine; spirobenzylisoquinoline; benzyloquinoline; etc. Volatile oils, steroids and flavonoids are found in Corydalis. The content of flavonoids and polyphenol was greater than that of alkaloids.

Numerous alkaloids, flavonoids, saponins, and terpenoids are present in Fumaria. Phthalide isoquinolines (including secophthalide isoquinoline), protoberberines, and spirobenzylisoquinolines are the major alkaloids, followed by benzylisoquinolines, aporphines, benzophenanthridines, indenobenzazepines, protopines, etc. The structural types of Hypecoum isoquinoline alkaloids can be divided into protoopioid, protoberberine, benzophenanthridine, aporphine, simple isoquinoline, benzyloquinoline, spirobenzylisoquinoline, etc. Other alkaloids such as benzazepines, phenylpropanamides, phthalidines and phenylethylamines were also identified. Protoopioid alkaloids are not abundant in this genus, but they are widely distributed. Simple isoquinoline, benzylisoquinoline and spirobenzene isoquinoline alkaloids are the most in Hypecoum, followed by aporphine and benzophenanthridine. Different from other genera of Papaveraceae, phthalide alkaloids are less reported in Hypecoum.
**Papaveroidae: Papaver, Meconopsis, and Argemone**

The genus *Papaver* is closely related with *Meconopsis* (blue poppy), and *Argemone* is basal to these genera in the subfamily Papaveroidae (Appendix 1). Isoquinoline alkaloids are chemical markers and bioactive constituents of *Papaver* species. (S)-reticuline is a pivotal intermediate in the biosynthesis of many BIAs among which berberine is present in species across the entire Ranunculales. The benzophenanthridine BIA, which includes the antimicrobial sanguinarine, is specific to the Papaveraceae family, whilst the phthalideisoquinoline noscapine and morphinan BIAs are exclusive to *Papaver* (opium poppy).

The number of BIAs identified in *Meconopsis* is less than that of *Papaver*, but the structural type of the former could be as diverse as that of the latter (Appendix 5), and more flavonoids were identified in *Meconopsis* than BIAs. Berberine, protopine, chelerythrine, sanguinarine, coptisine, palmatine, magnoflorine, and galanthamine are identified in *Argemone mexicana* roots. Papaver species.

**Papaveroidae: Chelidonieae, Glaucium and Eschscholtzia**

The genera *Eomecon*, *Sanguinaria* (bloodroot), *Macleaya*, *Glaucium*, *Chelidonium* (greater celandine) and *Hylomecon* are included in the tribe Chelidonieae. *Glaucium* is closer to *Chelidonium* and *Hylomecon* (Appendix 1), and *Macleaya* is basal to these 3 genera. *Eschscholtzia* is closer to *Eomecon* and *Sanguinaria* than to the above 4 genera. *Sanguinaria* contains benzophenanthridine and protopine BIAs at biologically relevant concentrations. Six types of BIAs were identified in *Eomecon*: 6 benzyltetrahydroisoquinolines, 9 protopines, 5 N-methyltetrahydroprotoberberines, 6 protoberberines, 8 benzophenanthridines, and 61 dihydrobenzophenanthridines. Benzo[c]phenanthridine and dihydrobenzo[c]phenanthridine BIAs, i.e., sanguinarine, chelirubine, macarpine, etc., were identified in *Eschscholtzia* and partially due to shared material basis. In the subfamily Berberidoideae, the genus *Berberis* is most commonly used in redness swelling and pain of the eye (TRI 4483, β 73), followed by gastroenteritis (TRI 3512, β 65), heat-clearing and detoxification (TRI 3114, β 59), dysentery (TRI 3430, β 56), carbuncle-abscess and sore-toxin (TRI 2604, β 54, Appendix 7), etc. Medicinal properties for all parts of *Berberis* plants include antioxidant, sedative, antimicrobial, anti-leishmaniasis, anti-herbicidal, antipyretic, anti-inflammatory, anti-arrhythmic, anti-cholinergic, cholagogic, anti-malaria. The main compounds found in various species of *Berberis* are berberine and berbamnine. Lipid-lowering and insulin-resistance improving actions are the most studied properties of berberine in numerous randomized clinical trials. There are also clinical trials regarding cardiovascular, anticaner, gastrointestinal, central nervous system (CNS), and endocrine effects (Appendix 8).

**Eunolopoeideae**

Nortriterpene and pentacyclic triterpene oligoglycosides were isolated from fresh leaves of *Euptelea polyandra*.

**Eupanolomematolga and Pharmacological Activity**

Each taxonomic group has distinctive bioactivities and ethnopharmacological uses, depending on their diverse phytochemicals. During the past decades, more than 30,000 cards have been collected, which record the ethnomedicinal uses of more than 5,000 Chinese medicinal plant species. The traditional remedy index (TRI) = C1/C2 × 100. C1 is the number of cards on which a specific ethnopharmacological use is recorded in China for a single genus; C2 is the number of species that has the same ethnomedicinal use in the same genus. TRI >300 is considered as significant. The higher the TRI, the more reliable that the genus has the specific ethnomedicinal use. Distribution density of ethnomedicinal use (β) = SP1/SP2 × 100, SP1 = C1, SP2 = number of Chinese species in this genus.

**Berberidaceae**

The ethnomedicinal use of Ranunculaceae has been evaluated. Berberidaceae is phylogenetically close to Ranunculaceae; accordingly, some genera of 2 families share analogous ethnomedicinal uses, partially due to shared material basis. In the subfamily Berberidoideae, the genus *Berberis* is most commonly used in redness swelling and pain of the eye (TRI 4483, β 73), followed by gastroenteritis (TRI 3512, β 65), heat-clearing and detoxification (TRI 3114, β 59), dysentery (TRI 3430, β 56), carbuncle-abscess and sore-toxin (TRI 2604, β 54, Appendix 7), etc. Medicinal properties for all parts of *Berberis* plants include antioxidant, sedative, antimicrobial, anti-leishmaniasis, anti-herbicidal, antipyretic, anti-inflammatory, anti-arrhythmic, anti-cholinergic, cholagogic, anti-malaria. The main compounds found in various species of *Berberis* are berberine and berbamnine. Lipid-lowering and insulin-resistance improving actions are the most studied properties of berberine in numerous randomized clinical trials. There are also clinical trials regarding cardiovascular, anticancer, gastrointestinal, central nervous system (CNS), and endocrine effects (Appendix 8).

The genus *Mahonia* is most commonly used in redness swelling and pain of the eye (TRI 3553, β 100), followed by dysentery (TRI 3095, β 100), tuberculosis (TRI 2625, β 89), gastroenteritis (TRI 2579, β 94), heat-clearing and detoxification (TRI 2178, β 61). The isolated compounds and crude extracts of *Mahonia* exhibit a wide spectrum of in vitro and in vivo
pharmacological effects, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antimutagenic and analgesic properties. Preparations containing Mahonia species exert good efficacy for the clinical treatment of dysentery, internal and external hemorrhage, acne vulgaris and chronic pharyngitis.

In the subfamily Nandinoideae, the genus Caulophyllum is traditionally used in promoting blood circulation to remove stasis (TRI 908, β 100), dispelling wind and dampness (TRI 582, β 100), and arthritis (TRI 736, β 100). The Caulophyllum extracts and compounds showed the antibacterial activity, anti-inflammatory and analgesic effects, antioxidant effects, antiacetylcholinesterase activity, effect on atherosclerosis and myocardial ischemia, antitumor activity, cytochrome p450 inhibition, topoisomerase inhibition, and effect on wound healing.420

The genus Nandina is used in heat-clearing and detoxification (TRI 405, β 100), cough suppression (TRI 605, β 100), bronchitis (TRI 405, β 100), and pertussis (TRI 845, β 100). Nandina extract and compounds showed the antibacterial, antitumor and anti-inflammatory effects.110-111 Caffeoyl glucosides of N. domestica inhibit lipopolysaccharide (LPS)-induced endothelial inflammatory responses.112-113 The essential oil of Nandina exhibited significant antioxidant activities.112

In the subfamily Podophyllidoideae, the genus Diphylleia is used in carbuncle-abscess and sore-toxin (TRI 500, β 100) and arthritis (TRI 500, β 100). Deoxypodophyllotoxin of Diphylleia and Podophyllum induced apoptosis of human prostate cancer cells.114 The podophyllotoxin of Diphylleia and Podophyllum could be the major antibacterial lignan,115 while flavonoids could be responsible for the antioxidant activity.

The genus Dysosma is most commonly used in carbuncle-absscess and sore-toxin (TRI 2869, β 100), followed by snakebite (TRI 2144, β 87), blood-activating and stasis-removing (TRI 1694, β 87), mumps (TRI 903, β 75), dispersing swelling and resolving toxin (TRI 574, β 87). Podophyllotoxins and flavonoids have cytotoxic activities.116 Deoxypodophyllumtoxin had antineoplastic effects on glioblastoma U-87 MG and SF126 cells.117 Kaempferol of Dysosma versipellis inhibits angiogenesis through vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) pathways.118 Flavonol dimers from callus cultures of D. versipellis display in vitro neuraminidase inhibitory activities.119 The coexisting flavonoids alleviate the podophyllotoxin toxicity.120

The genus Sinopodophyllum is used in cough suppression (TRI 357, β 100), traumatic injury (TRI 514, β 100), stomachache (TRI 514, β 100), arthritis (TRI 357, β 100), and menoxenia (TRI 357, β 100). Flavonoids of Sinopodophyllum have anticancer activities against human breast cancer cells.225 S. hexundrum promotes K562 cell apoptosis possibly by inhibiting BCR/ABL-STAT5 survival signal pathways and activating the mitochondrion-associated apoptotic pathways.123

The genus Epimedium is most commonly used in impotence (TRI 2597, β 100), followed by strengthening yang (TRI 1988, β 70), dispelling wind and dampness (TRI 1838, β 70), arthritis (TRI 1062, β 72), and neurasthenia (TRI 662, β 80). Epimedium and its active compounds displayed wide pharmacological activities, e.g., strengthening yang, hormone regulation, anti-osteoporosis, immunological function modulation, anti-oxidation and anti-tumor, anti-aging, anti-atherosclerosis and anti-depressant activities.124 The total flavonoid fraction of the E. koreanum extract had neuroprotective effects on dopaminergic neurons.125 Icarin had neuroprotective properties in the mouse model of Parkinson's disease.126 The protective mechanisms of icariin against oxygen-glucose deprivation-induced injury may be related to down-regulating the expression of hypoxia-inducible factor 1α (HIF-1α), heat shock protein 60 (HSP-60) and HSP-70.127 Epimedium improves neuroplasticity and accelerates functional recovery of the diseased brain.128

The genus Plagiorhegma (Jeffersonia) is used in dysentery (TRI 400, β 100) and redness swelling and pain of the eye (TRI 400, β 100). The protoberberine BIA jatrorhizine, the lignane glucosides dehydrodiconiferyl-alcohol-4-β-D-glucoside and its isomer dehydrodiconiferyl-alcohol-γ-β-D-glucoside, isolated from Plagiorhegma dubium cell culture, were anti-inflammatory.129 The extract from Jeffersonia dubia exhibited the high cytotoxicity in vitro,130 and suppressed the LPS-induced nitric oxide (NO) production.

Menispermeaceae

In the subfamily Tinosporoideae, the genus Arcangelisia is used in heat-clearing and detoxification (TRI 320, β 100), dysentery (TRI 500, β 100), gastroenteritis (TRI 320, β 100), and malaria (TRI 500, β 100). N-trans-feruloyltyramine of Arcangelisia gusanlung is an active phenylpropanoid compound. It possesses antioxidant, antimicrobial, anti-melanogenesis, immunomodulative, and antitumor activities.131 Gusanlungiosides A–D, the megastigmane glycoside from the stems of A. gusanlung, are potential tyrosinase inhibitors.132 Berberine, extracted from A. flava, dose-dependently inhibited Plasmodium telomerase activity.133 A. flava and Fibraurea tintoria have antiplasmodial activity.134 Protoberberine BIAs and 20-hydroxyecdysone of A. flava had the antibabesial activity against Babesia gibsoni in culture.135

As compared with Arcangelisia, Tinospora has more diverse chemical components (Appendix 5) and is useful as the anti-oxidant, anti-hyperglycemic, anti-hyperlipidemic, hepatoprotective, cardiovascular protective, neuroprotective, osteoprotective, radioprotective, anti-anxiety, adaptogenic, analgesic, anti-inflammatory, antipyretic, thrombolytic, anti-diarrheal, anti-ulcer, antimicrobial and anticancer agents.536 Its anti-diabetic, antipyretic, anti-inflammatory, anti-oxidant, hepato-protective, and immuno-modulatory activities are
prominent. The genus Fibraurea is most commonly used in heat-clearing and dampness-drying (TRI 500, β 100), and carbuncle-abscess and sore-toxin (TRI 500, β 100), followed by dysentery (TRI 417, β 100), sore throat (TRI 417, β 100), redness swelling and pain of the eye (TRI 417, β 100), heat-clearing and detoxification (TRI 320, β 100). The cognitive-enhancing effects and antidepressant effect of Fibraurea alkaloids are noteworthy. The aporphine BIA roemerine from the fresh rattan stem of Fibraurea racisa has antifungal activity. The methanol-water fraction of F. tinctoria showed higher anti-proliferative activities than its methanolic extract.

In the subfamily Menispermoideae, the genus Cocculus is most commonly used in dispelling wind and dampness (TRI 1521, β 100), followed by arthritis (TRI 1108, β 100), carbuncle-abscess and sore-toxin (TRI 720, β 100), snakebite (TRI 637, β 100), heat-clearing and detoxification (TRI 605, β 100). Extracts of Cocculus have antimicrobial, antiabetic, immunomodulatory and hepatoprotective activities.

The genus Cyclea is most commonly used in heat-clearing and detoxification (TRI 847, β 83), followed by sore throat (TRI 476, β 83), carbuncle-abscess and sore-toxin (TRI 408, β 50), snakebite (TRI 400, β 67) and urinary tract infection (TRI 313, β 50). (–)-Curine, a BBI alkaloid from the roots of Cyclea waltii, induced cell cycle arrest and cell death in hepatocellular carcinoma cells in a p53-independent way. Tetrandrine isolated from Cyclea peltata induced cytotoxicity and apoptosis through reactive oxygen species (ROS) and caspase pathways in breast and pancreatic cancer cells. The roots of Cissampelos pareira var. hirsuta are used in the treatment of various diseases like stomach pain, fever, skin disease, etc. in Ayurveda and is commonly known as Patha. Two other species, Cyclea peltata and Stephania japonica of the same subfamily are being used as the source of Patha in various parts of India.

The genus Stephania is used in heat-clearing and detoxification (TRI 4102, β 94) and dispelling wind and dampness (TRI 1945, β 44). S. tetrandra has a wide range of pharmacological properties, e.g., antimicrobial, anti-inflammatory, anticancer/antiproliferative, CNS activity, cardiovascular activity, immunomodulatory, antifibrotic, antiabetic, antiplatelet effects, etc. So far, studies focus principally on the alkaloids and extracts of Stephania. The most commonly reported molecules are tetrandrine, fangchinoline and cepharanthine. Cepharanthine suppresses nuclear factor-kappa B (NF-κB) activation, lipid peroxidation, NO production, cytokine production, and expression of cyclooxygenase; it could be used against severe acute respiratory syndrome (SARS)-coronavirus (CoV)-2 and homologous viruses, and could be therapeutically important in managing coronavirus disease (COVID)-19 cases.

The genus Diploclisia is used in urinary tract infection (TRI 320, β 100), arthritis (TRI 320, β 100), and snakebite (TRI 500, β 100). D. glaucescens showed strong antifeedant activity. The scarcity of pharmacological data suggests the asymmetry between phytochemical research, ethnomedicinal experiences and pharmacological research.

The genus Menispermum is used in heat-clearing and detoxification (TRI 1108, β 100) and sore throat (TRI 931, β 100). The M. dauricum alkaloids exhibited significant anti-hypoxia activity. Oxoisooprinone of M. dauricum potent telomerase inhibitor. Phenolic alkaloids from M. dauricum inhibits BxPC-3 pancreatic cancer cells by blocking of Hedgehog signaling pathway. They also inhibited gastric cancer in vivo. The closely related genus Sinomenium is used in dispelling wind and dampness (TRI 514, β 100) and arthritis (TRI 514, β 100). The top 5 predicted significant pathways of S. acutum targets include phosphatidylinositol 3 kinase/Akt (PI3K/Akt) signaling, prostate cancer signaling, macrophage migration inhibitory factor (MIF) regulation of innate immunity, guanosine-binding protein coupled receptor (GPCR) signaling, and ataxia telangietasia mutated protein (ATM) signaling. The mitogen activated protein kinase 1 (MAPK1), MAPK3, p65 nuclear factor κ B (RELA), nuclear factor of κ B inhibitor alpha (NFκBIA), interleukin 1 β (IL-1β), prostaglandin G/H synthase 2 (PTGS2) and tumor protein 53 (TP53) were the predicted critical targets in various diseases treated with S. acutum. Sinomenine, a main compound from S. acutum, showed the activities on the immune system, cardiovascular system, and nervous system.

Lardizabalaceae and Circiaeerataceae

There is a lack of various types of alkaloids in Lardizabalaceae, and the ethnomedicinal uses and pharmacological activities of this family are distinct when compared with the above 3 families. In the subfamily Sargentodoxeae, the genus Sargentodoxa is most commonly used in promoting blood circulation to remove stasis (TRI 2181, β 100), followed by dispelling wind and dampness (TRI 1706, β 100), arthritis (TRI 1290, β 100), traumatic injury (TRI 632, β 100), menoxenia (TRI 632, β 100). The extract and compounds of S. cuneata have a wide spectrum of pharmacological activities, including antimicrobial, antitumor, anti-inflammatory, antioxidants, anti-sepsis and anti-arthritis effects, as well as protective activity against cerebrovascular diseases.

In the subfamily Lardizabalidaeae, the genus Akebia is used in promoting blood circulation to remove stasis (TRI 455, β 100), heat-clearing and dampness-drying (TRI 368, β 100), arthritis (TRI 655, β 100), and urinary tract infection (TRI 455, β 100). Bioactivity studies of A. quinata stem, leaf and/or fruit extracts confirmed diuretic, hepatoregenerative, neuroprotective, analgesic, anti-inflammatory, and anti-obesity effects and an influence on ethanol metabolism. A. trifoliata stem, leaf and/or fruit extracts had different action profiles. Both species showed the antibacterial and anticancer (liver and stomach) effects.

The genus Holboellia is most commonly used in arthritis.
(TRI 800, β 100), followed by urinary tract infection (TRI 613, β 100), beriberi (TRI 613, β 100), breast milk stoppage (TRI 450, β 100), menoxenia (TRI 313, β 100). There is a lack of pharmacological studies of triterpenoid saponins from Holboellia. The phylogenetically close genus Stauntonia is used in arthritis (TRI 613, β 100) and traumatic injury (TRI 514, β 75). Stauntonia extract and triterpenoid showed anti-inflammatory and anti-prostate hyperplasia effects. It is speculated that the triterpenoids of Holboellia also have anti-inflammatory activity. S. hexaphylla leaf constituents inhibit lens aldose reductase and the formation of advanced glycation end products. Triterpenoid saponins from S. chinensis ameliorate insulin resistance via the AMP-activated protein kinase (AMPK) and IR/IRS-1/PI3K/Akt pathways in insulin-resistant HepG2 cells. S. chinensis polysaccharide showed protective effect on CCl4-induced acute liver injuries in mice. Nor-oleanane triterpenoid saponins from S. brachyanthera inhibit uridine diphosphate (UDP)-glucuronosyltransferases, and had anti-gout activity.

**Papaveraceae**

In the subfamily Fumarioideae, around 428 species of genus Corydalis are distributed worldwide, 298 of which are in China, and 10 groups and 219 species are endemic in China. Corydalis is widely used as folk medicines in China and adjacent countries, especially in traditional Tibetan medicines, for the treatment of fever, hepatitis, edema, gastritis, cholecytitis, hypertension and other diseases. According to Chinese Dictionary of Ethnic Medicine, up to 62 Corydalis species are used by Chinese ethnic minorities against musculoskeletal diseases, followed by 49 species against respiratory diseases, 42 species against skin diseases, 35 against gastrointestinal diseases, 32 against circulatory diseases and hepatobiliary diseases, respectively. Rhizoma Corydalis has antiinocceptive, sedative, anti-epileptic, antidepressive and anti-anxiety, acetylcholinesterase inhibitory effect, drug abstinenence, anti-arrhythmic, antymyocardial infarction, dilated coronary artery, cerebral ischaemia reperfusion (I/R) injury protection, antihypertensive, anti-thrombosis, antithrombotic enterogastrointestinal ulcer, liver protection, antimicrobial, anti-inflammation, antiviral, and anticancer effects.

According to Chinese Dictionary of Ethnic Medicine, both Fumaria officinalis and F. schleicheri are used by Chinese ethnic minorities against skin diseases and the former is also used against gastrointestinal and urinary ailments. Pharmacological studies revealed diverse bioactivities, e.g., hepatoprotective, anti-inflammatory, antimicrobial, antioxidant, and anti-tumor activities. F. indica (Fumitory) showed various pharmacological activities, e.g., smooth muscle relaxant, spasmyogenic and spasmyolytic, analgesic, anti-inflammatory, neuropharmacological, hepatoprotective, antifungal and antibacterial activities.

**Hypecoum erectum and H. leptocarpum** are used by Chinese ethnic minorities against poisoning, gastrointestinal/ hepatobiliary diseases, eye/ear/nose/throat diseases, musculoskeletal diseases, respiratory diseases and skin diseases. Isoquinolines of H. erectum had anti-inflammatory and analgesic activities. The crude alkaloid mixture of H. ponticum containing quaternary isoquinolines showed potent antifungal and antibacterial activity. Leptopidine of H. leptocarpum could suppress growth and induce cytotoxicity in breast cancer cells via inhibiting fatty acid synthase expression.

In the subfamily Papaveroideae, Glauccium squamigerum is used by Chinese ethnic minorities against gastrointestinal and respiratory diseases. The most reported bioactivity of Glauccium alkaloids is anticancer and anti-cholinesterase effects. Yet, most species have not been investigated either phytochemically or pharmacologically. G. flavum (yellow horn poppy) is the most studied species. In the tribe Chelidonieae, Chelidonium majus exhibits anti-inflammatory, antimicrobial, antitumor, analgesic, and hepatoprotective effects supporting its traditional uses. However, several important properties, e.g., diuretic, antitussive and eye-regenerative effects, have not been scientifically studied. C. majus also has scientifically proven effects, such as anti-osteoporotic activity and radioprotection, which are not recorded in traditional medicine. In addition, the hepatoprotective versus hepatotoxic effects of C. majus deserve attention.

**Macleaya cordata** and **M. microcarpa** are used by Chinese ethnic minorities against poisoning, gastrointestinal, musculoskeletal, oral/ear/nose/throat, pediatric, reproductive, skin and respiratory diseases. Macleaya compounds and/or extract have antitumor, anti-inflammatory, insecticidal, and antibacterial activities in addition to potential toxicities. Historical uses of Sanguinaria can provide valuable information for the research and development of new therapies. S. canadensis alkaloids have the anticancer, cardiovascular, anti-inflammatory, anti-infectious, neurotransmitter, local anesthetic, gastrointestinal and coagulation effects. Eomecon chionantha is used by Chinese ethnic minorities against poisoning, gastrointestinal, eye, musculoskeletal, oral/ear/nose/throat, pediatric, skin and respiratory diseases. The pharmacological study of Eomecon is lacking.

**Argemone mexicana** has antibacterial, anti-cancer, sedative and probably anti-anxiety properties. A. mexicana BIAs have antimicrobial, antiparasitic, antimalarial, pesticide, cytotoxic and neurological properties. A. mexicana silver nanoparticles are suitable for bio-formulation against mosquitoes and microbes. Alkaloids isolated from A. mexicana are cytotoxic to the SW480 human colon cancer cell line. According to Chinese Dictionary of Ethnic Medicine, up to 19 Meconopsis species are used by Chinese ethnic minorities against respiratory diseases, followed by 18 species against hepatobiliary diseases, 12 against musculoskeletal diseases, 9 against ear/nose/throat diseases, 8 against skin diseases. Meconopsis plants have antitumor, hepatoprotective, analgesic, antimicrobial, anti-oxidant, antitussive, and anti-inflammatory activities. The crude extracts and metabolites of Meconopsis also showed CNS effects, cardiovascular
effects, antibiosis, and antiviral activity.\textsuperscript{(173)} Six Papaver species are used by Chinese ethnic minorities against respiratory, gastrointestinal, musculoskeletal, reproductive and skin diseases.\textsuperscript{(5)} Papaver species have analgesic, antioxidant, antimutagenic, anticarcinogenic, antiproliferative, antiviral, and cardioprotective activities.\textsuperscript{(6)} The pharmacological study of Pteridophyllum is lacking.

**Eupteleaceae**

Nortriterpene oligoglycosides, isolated from the fresh leaves of Euptelea polyandra, have gastroprotective activity.\textsuperscript{(174)} E. polyandra extracts exhibited pro-osteoblastic and anti-osteoclastic activity with low cytotoxicity,\textsuperscript{(175)} suggesting their potential effectiveness against osteoporosis.

**DISCUSSION**

**Relationship between Molecular Phylogeny and Chemical Profile of Ranunculaceae Families**

Phylogenetically, Ranunculales is sister to all other eudicots (Appendix 2), and there is some uncertainty about basal eudicot relationship. The phytochemical, metabolomic and chemotaxonomic studies of 7 Ranunculaceae families may help resolve the interrelationship of basal eudicots.\textsuperscript{(176)} Ranunculaceae is the largest Ranunculaceae family with more than 50 genera and more than 2,000 species, followed by Papaveraceae, Berberidaceae, Menispermaceseae and Lardizabalaceae, which lead to a significant chemodiversity. The commonly found phytometabolites, e.g., flavonoids, phenolics, terpenoids, steroids and organic acids, etc. (Appendix 5), are also identified from Ranunculaceae families. Though, the most striking phytometabolites of Ranunculaceae are various alkaloids, especially diverse types of BIAs.

BIAs can be used as the chemotaxonomic marker, which consist of more than 2,500 diverse structures mainly generated by the order Ranunculales and Eumagnolliids. BIAs are also present in Rutaceae, Lauraceae, Cornaceae and Nelumbonaceae,\textsuperscript{(177)} and sporadically distributed throughout the order Piperales. The limited occurrence of BIAs outside Ranunculaceae and Eumagnolliids suggests the requirement for a highly specialized, but evolutionarily unstable cellular platform to accommodate or reactivate the BIA biosynthesis pathway in divergent taxa. Some BIAs function in the defense of plants against herbivores and pathogens, thus the BIA biosynthesis might play an important role in the reproductive fitness of certain plants; more importantly, these defense molecules also find a place in the treatment of human diseases (Appendixes 7 and 8).

Large-scale molecular phylogenies provide a framework for interdisciplinary investigations in chemotaxonomy and evolution of phytometabolite biosynthesis,\textsuperscript{(178)} equally importantly, the phylogenetic signal of specific medicinal compounds and/or therapeutic efficacies could be identified.\textsuperscript{(179)} It is obvious that different types of BIAs are distributed selectively. Aporphine BIAs and protoberberine BIAs are most widely distributed, and are abundant in most genera of Menispermaceseae and Papaveraceae. However, unlike Ranunculaceae, the aporphine BIA magnoflorine was not detected in Papaveraceae genera such as Meconopsis, Macleaya, Corydalis and Fumaria.\textsuperscript{(178)} Berberine is abundant in Ranunculaceae subfamilies Thalictrioideae and Coptidoideae, Berberidaceae, but absent in Papaveraceae genera except Chelidonium and Macleaya.\textsuperscript{(114)} The protopine BIAs and phenanthridine BIAs are mainly distributed in Papaveraceae, morphinan BIAs and benzyltetrahydroisoquinoline alkaloids are concentrated in some genera of Menispermaceseae and Papaveraceae, while BBIs are found in Menispermaceseae, Berberis and Mahonia of Berberidaceae, Isopyrum and Dichocarpum of Ranunculaceae.\textsuperscript{(66,179)} The data about the distribution of pavinane alkaloid, quinolizidine alkaloid, pyrrolidine alkaloid and steroidal alkaloid in Ranunculaceae are relatively few, and their distribution pattern is uncertain. In contrast, alkaloids with certain distribution could be useful in chemotaxonomy, and the distribution patterns provide clues for finding new medicinal compounds purposefully, not just by chance.

Besides alkaloids, steroids, phenolics, flavonoids and their glycosides are scattered in various Ranunculaceae families (Appendix 5). Lignans are commonly found in Ranunculaceae families except Papaveraceae. The pentacyclic triterpenoids are distributed throughout Ranunculaceae, which is relatively devoid of diterpenoids and tetracyclic triterpenoids. Some of these compounds have a variety of pharmacological activities (Appendix 8) and the relevant families and genera should be further excavated.

It was suggested that, from the perspective of pharmacophylogeny, Berberidaceae could be treated as 4 independent families: Nandinaeeae, Berberidaceae (s.s.), Podophyllaceae and Leonticeae.\textsuperscript{(15)} The monotypic family Nandinaeeae is rich in various BIAs, e.g., aporphine BIA, protoberberine BIA, protopine BIA, morphinan BIA and benzyltetrahydroisoquinoline alkaloid, which is similar to that of Menispermaceseae and Papaveraceae, but BBI, phenanthridine BIA and pavinane alkaloid are absent in Nandina. The presence of the cyanogenic compound nandinin, biflavonoid amentoflavone and benzaldehyde-4-O-glucoside in Nandina indicates its relatively distant relation with the other Berberidaceae genera. Nandina indica is ethnopharmacologically used as a medicament for clearing heat and counteracting toxins, and as antitussive (Appendix 7). The proposed Berberidaceae (s.s.) consists of Berberis and Mahonia, containing mainly BIAs, e.g., aporphine BIA, protoberberine BIA and BBI, particularly a higher content of type \( \text{BBI oxyacanthine} \). Ethnopharmacologically the plants of Berberis and Mahonia are mainly used as medicines for clearing heat and counteracting toxins. Plants of both genera have long been used as the main sources of berberine and berberine,\textsuperscript{(180,181)} The proposed Podophyllaceae can be divided into 2 tribes. The tribe Podophylleae, consisting of Podophyllum, Sinopodophyllum, Dysosma and Diphyylea, is rich in various podophyllotoxin lignans, and these plants are used as the most important source for producing the anticancer
podophyllotoxin’s derivatives. Ethnopharmacologically, these plants are mainly used for activating blood, revolving stasis, relieving swelling, removing toxin, and clearing heat. The tribe Epimieae, consisting of Epimedium, Vancouveria, Jeffersonia and Plagiorhegma, has diverse chemicals. Both Epimedium and Vancouveria contain predominantly icarin flavonoids, the characteristic chemical of this group. Ethnopharmacologically the Epimedium plants are used as a male sexual tonic, and for dispelling wind and removing dampness. The phytochemistry of Achiys, Jeffersonia and Ranzania has not been thoroughly investigated. Jeffersonia dubia is used for dysentery and inflammatory eye pain by the Korean nationality of Northeast China. The proposed Leonitaceae, including Gymnospermium, Leontice, Caulophyllum and Bongardia, contains mainly β-amyrin triterpenoids and quinolizidine alkaloids; they are used for activating blood, revolving stasis, dispelling wind and removing dampness.

### Relationship between Phytometabolites and Therapeutic Efficacy of Ranunculales Families

Among the traditional medicinal uses of Ranunculales, 25 genera, including 8 of Ranunculaceae, 5 of Berberidaceae and Papaveraceae respectively, 4 of Lardizabalaceae, and 3 of Menispermaceae, are used to treat arthritis (Appendix 7). Up to 29 genera, i.e., 13 of Papaveraceae, 4 of Berberidaceae, and 3 of Menispermaceae, are used to treat carbuncle-abcess and sore-toxin. Twenty-six genera, including 11 of Ranunculaceae, 9 of Papaveraceae, 4 of Berberidaceae, and 2 of Lardizabalaceae, are used to treat traumatic injury. Twenty-three genera of Ranunculaceae, Menispermaceae and Berberidaceae, rather than Lardizabalaceae and Papaveraceae genera, are used in heat-clearing and detoxification; 16 genera of Ranunculaceae, Papaveraceae, Berberidaceae and Menispermaceae, rather than Lardizabalaceae genera, are ethnomedicinally used against dysentery. The greater differences of ethnopharmacological uses between Ranunculaceae and other Ranunculales families are revealed in this study (Appendices 7 and 9). In addition to the above 5 common uses, there are many plants of Papaveraceae, Berberidaceae, Menispermaceae and Lardizabalaceae used to treat snake bites, gastroenteritis, redness swelling and pain of eyes, sore throat and urinary tract infection; in contrast, there are more Ranunculaceae plants used in wind-dispersing and dampness-eliminating, blood-activating and stasis-removing, swelling-reducing and detoxification, or as the pesticide, antitussive and expectorant. Such differences in traditional uses could be partially explained by the distinct material basis of different families/genera (Appendices 4 and 5). Since the material basis and responsible phytometabolites of some ethnomedicinal utilities are still elusive, it is imperative to further clarify the internal relationship between the two, so as to better harness the traditional wisdom in novel drug discovery and development.

In modern bioactivity studies, 46 genera, including 17 of Ranunculaceae, 12 of Papaveraceae, 8 of Menispermaceae, 7 of Berberidaceae, 2 of Lardizabalaceae, showed the anticancer/cytotoxic/antiproliferative activities in vitro or in vivo (Appendix 8). Up to 39 genera, i.e., 15 of Ranunculaceae, 12 of Papaveraceae/Eupteleaceae, 5 of Berberidaceae, 4 of Menispermaceae, and 3 of Lardizabalaceae, displayed the antibacterial/antifungal/antiviral properties, which is followed by anti-inflammatory (36 species), analgesic/sedative (24 species), immunomodulatory and antioxidant (18 respectively), hepatoprotective and antiparasitic/pesticide activities, etc. In addition, 6 Ranunculaceae species exhibited the antihypertensive activity, whereas 9, 8 and 7 species of other 4 families had the anti-diabetic, neuroprotective, anti-atherosclerosis and myocardial ischemia properties, respectively. Some Ranunculaceae alkaloids, terpenoids, saponins and polysaccharides have shown anticancer activities in vitro and in vivo. Most concerns have been raised for the monoterpene thymoquinone and the isoquinoline alkaloid berberine, and the latter could also partially explain the anticancer properties of Berberidaceae plants. The anticancer compounds of other 3 families are also diverse. For example, both oxoisoaoporphine alkaloids and BBIs of Menispermaceae have anticancer effects (Appendix 9). The pentacyclic triterpene saponins of Lardizabalaceae such as Akebia saponin E inhibited the proliferation of carcinoma cells. The phanethrinine BIAS of Papaveraceae such as sanguinarine and chelerythrine had strong anticancer activities. At least 17 Ranunculaceae genera are rich in anticancer phytometabolites, and 29 genera of other Ranunculales families have numerous cytotoxic compounds, constituting an enormous chemical space for bioprospecting. Some of these phytometabolites induce the cell cycle arrest and apoptosis of cancer cells or enhance immune activities, while others inhibit the proliferation, invasion, angiogenesis and metastasis, or reverse the multi-drug resistance of cancer cells, thereby regulating hallmarks of cancer. These phytometabolites could exert their anticancer activities via multiple signaling pathways.

### Conclusion

The 7 families of Ranunculales are rich in medicinal species frequently utilized by traditional medical systems and ethnomedicine, which represent a treasure chest of biodiversity and chemodiversity. The phylogenetically related species often have similar phytometabolites, and therefore similar bioactivities. This study gives a systematic review of the molecular phylogeny, chemical components and pharmacological activities of Ranunculales within the framework of pharmacophenylogy. The metabolomic techniques greatly facilitate the systematic mining of both primary and secondary metabolites; the far-reaching metabolite profiling based on multiple analytical platforms enabled a more inclusive picture of overall metabolism occurring in selected species. Traditionally important medicinal species and their close relatives should be subjected to a metabolomics approach.

The surging bioactivity studies of extracts and compounds help clarify the rationale of ethnomedicinal utilities and deepen our understanding of the medicinal potential of Ranunculales.
The most studied compounds of this order include BIAs, flavonoids and phenolics, lignans, terpenoids and saponins, etc. BBI alkaloids, with versatile bioactivities, are especially abundant in Berberidaceae and Menispermaceae. The most frequent ethnomedicinal uses are arthritis, heat-clearing and detoxification, carbuncle-abscess and sore-toxin, etc. The most studied bioactivities are anticancer/cytotoxic, antimicrobial, and anti-inflammatory activities. Some pharmacological activities are in line with traditional uses, but some others are not related to the latter, implying the broad pharmacological space to be explored. Our pharmacophylogeny analysis, integrated with both traditional and contemporary medicinal uses, agrees with the molecular phylogeny based on chloroplast and nuclear DNA sequences. With the increasing evidence of chemotaxonomy and therapeutic efficacy of each taxonomic group, the underlying connection between phylogeny, chemodiversity and clinical uses is revealed, which facilitate the conservation and sustainable utilization of Ranunculales pharmaceutical resources, as well as developing novel plant-based pharmacotherapy. The high-quality chromosome-scale genome assembly and annotation of Coptis chinensis have been presented.\(^{(190)}\) In the near future, the whole genome sequences of more Ranunculales species could be deciphered; it will be possible to reconstruct the phylogenetic relationship based on transcriptome and/or genome sequences. Decoding the biosynthesis pathways and their regulation based on genome sequences could be conducive to predicting the specific types or classes of compounds, and cross-validating the metabolomic data. With the development of R language software packages, the distribution of therapeutic compounds and efficacy of Ranunculales species on the seed plant Tree of Life\(^{(5,6,191)}\) can be quantitatively investigated.\(^{(14,187)}\)

The absorption, distribution, metabolism, and excretion/toxicity (ADMET) properties of phytometabolites have the significant impact on the therapeutic efficacy.\(^{(186)}\) The structurally analogous compounds could have similar (or distinct) metabolism and pharmacokinetic attributes. In the early stage of drug discovery and development, the ADMET properties should also be taken into account in the context of pharmacophylogeny, so as to improve the theoretical basis and application value of pharmacophylogeny. In the mechanism studies, the gene expression profiling and relevant omics platforms (e.g. genomics, transcriptomics, proteomics, and metabolomics) could reveal differential effects of phytometabolites on the phenotypically heterogeneous human cells.

Conflict of Interest

The authors declare that they have no competing interests. Author XIAO Pei-gen is a member of the Editorial Board for CJIM. The paper was handled by the other Editor and has undergone rigorous peer review process. Author XIAO Pei-gen was not involved in the journal’s review of, or decisions related to, this manuscript.

Author Contributions

Xiao PG, Hao DC and Xu LJ contributed to study concept and design. Hao DC undertook data analysis and interpretation and wrote the paper. Xu LJ provided financial support. Hao DC and Xu LJ contributed equally to this work. Zheng YW and Lyu HY collected data and Zheng YW drew figures.

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