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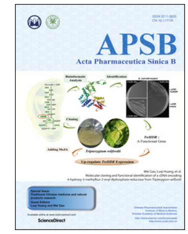

REVIEW

Anemone medicinal plants: ethnopharmacology, phytochemistry and biology

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Abstract The Ranunculaceae genus *Anemone* (order Ranunculales), comprising more than 150 species, mostly herbs, has long been used in folk medicine and worldwide ethnomedicine. Various medicinal compounds have been found in *Anemone* plants, especially triterpenoid saponins, some of which have shown anti-cancer activities. Some *Anemone* compounds and extracts display immunomodulatory, anti-inflammatory, antioxidant, and antimicrobial activities. More than 50 species have ethnopharmacological uses, which provide clues for modern drug discovery. *Anemone* compounds exert anticancer and other bioactivities *via* multiple pathways. However, a comprehensive review of the *Anemone* medicinal resources is lacking. We here summarize the ethnomedical knowledge and recent progress on the chemical and pharmacological diversity of *Anemone* medicinal plants, as well as the emerging molecular mechanisms and functions of these medicinal compounds. The phylogenetic relationships of *Anemone* species were reconstructed based on nuclear ITS and chloroplast markers. The molecular phylogeny is largely congruent with the morphology-based classification. Commonly used medicinal herbs are distributed in each subgenus and section, and chemical and biological studies of more unexplored taxa are warranted. Gene expression profiling and relevant "omics" platforms could reveal differential effects of phytometabolites. Genomics, transcriptomics, proteomics, and metabolomics should be highlighted in deciphering novel therapeutic mechanisms and utilities of *Anemone* phytometabolites.

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1. Introduction

Anemone is a genus of more than 150 species of flowering plants in the family Ranunculaceae, native to the temperate zones of both Northern and Southern hemispheres. It is closely related to *Pulsatilla*, *Clematis*, and *Hepatica* morphologically and phytochemically[1]. More than 50 species have ethnopharmacological uses, which provide clues for modern drug discovery. Some traditional claims of *Anemone* species have been validated scientifically by pre-clinical and clinical studies[2,3]. The state-of-the-art methods have been adopted to isolate bioactive chemical constituents from *Anemone* species following bioactivity-directed fractionation[4,5]. Some modes of action of bioactive extracts or compounds of *Anemone* species have been established[6,7]. However, a comprehensive review of the *Anemone* medicinal resources is lacking. In the present article, we summarize the ethnomedical knowledge and the recent progress in uncovering the chemical and pharmacological diversity of *Anemone* medicinal plants, as well as the emerging molecular machineries and functions of these medicinal compounds. Gaps are also pointed out and further work is suggested.

Exhaustive literature search in NCBI PubMed, Google, Bing, and CNKI (http://cnki.net/) has been performed to outline the progress of *Anemone* research during the last three decades. Search terms "anti-cancer", "anti-inflammatory", "antioxidant", "saponin", "triterpene", "polysaccharide", etc. were used, com bined with "*Anemone*" and the names of species.

2. Ethnopharmacology

More than 50 *Anemone* species are used in various traditional medical systems (Table 1, Fig. 1). Fifty-three species, 9 subspecies and 36 varieties are found in China, which are distributed in most provinces except Guangdong and Hainan. According to our field survey, at least 38 species/varieties have ethnopharmacological uses. In traditional Chinese medicine (TCM) and folk medicine, *Anemone* is used in heat-clearing and detoxification [traditional remedy index (TRI) 424, distribution density of ethnopharmacological use (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)[8]. Correspondingly, 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[9]. For instance, *Anemone raddeana*, distributed in Far East, is commonly used in Northeast China for rheumatism, arthritis, and skin infection, etc. Liang Tou Jian (Zhu Jie Xiang Fu in Chinese), the rhizome of *A. raddeana*, is recorded in *China Pharmacopoeia* 2015 version. *Anemone altaica*, extensively distributed in Europe and Northern Asia, is used in Northwest China for epilepsy, neurosis, and rheumatic arthralgia. The rhizome of *A. altaica* is sometimes called "Jiu Jie Chang Pu", but actually "Jiu Jie Chang Pu" originally referred to the rhizome of *Acorus tatarinowii* (Araceae), which has distinct therapeutic components[10]. *Anemone vitifolia* (wild cotton) of the pan-Himalaya region is used in Southwest China for traumatic injury, rheumatic arthralgia, enteritis, dysentery, and ascariasis, etc.

In Southwest China, *Anemone* has been undergoing rapid diversification following the uplift of Qinghai Tibet Plateau in the Quaternary Period and the emergence of "sky islands"[11]. Many alpine *Anemone* species are used in Tibetan medicine (Table 1)[12,13]. For instance, Suga is the ripe seed of *Anemone rivularis*, *A. rivularis var. floreminore*, *A. obtusiloba*, *A. obtusiloba ssp. ovalifolia*, and *A. demissa*, and is used in diuresis, detumescence, enriching blood, warming the body, wound healing, and pus drainage[13,14]. Burchin, the root and flower of *Anemone trullifolia* and *A. trullifolia var. linearis*, is used for muscle-relaxation, blood-activation, and as an antitussive. Suga Angbo, the root, fruit, and whole plant of *A. demissa*, *A. demissa var. major*, *A. demissa var. villosissima*, and *A. imbricata*, is efficient in anti-rheumatism, helping digestion, dysentery (whole plant), eliminating chill and dampness, dissolving masses, and detoxification (fruit).

Various *Anemone* species are also used in the ethnomedicine of India, Korea, Mongolia, America, and Europe, etc. (Table 1). For instance, *Anemone biflora* bulb is styptic and is applied on boils, burns, cuts and wounds as an antiphlogistic[15]. The root and leaves of *Anemone canadensis* was one of the most highly esteemed medicines of the Omaha and Ponca Indians (http://plants.for9.net/edible-and-medicinal-plants). A decoction of the root was used as an anthelmintic and to treat pain in the lumbar region. An infusion of the root was used as an eye wash to treat crossed eyes, twitches and eye poisoning. In Korean medicine, the rhizome of *Anemone amurensis* is useful in paralysis, menoxenia, stomach ache, and pertussis[16]. *Anemone stolonifera* and *A. nikoensis* are for edible use in East Asia.

3. Phytochemical components

Identified *Anemone* compounds include triterpenoids, saponins, steroids, lactones, fats and oils, saccharides, and alkaloids, etc[9,17,18]. Oleanolic acid triterpene saponin is abundant in *Anemone* species (Supplementary Table S1). *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.

Figure 1 (A) Habitat of *Anemone*; (B) whole plant and flower of *Anemone rivularis*, taken in the Alpine Botanic Garden of Shangri-La, Yunnan, China; (C) fruit of *Anemone*, taken by the side of the Ni Yang river, Tibet, China.

Table 1 Ethnopharmacological uses of *Anemone* species.

Species	Medicinal part	Therapeutic efficacy	Distribution	Note
A. altaica	Rhizome	Tranquilizing, orifice-opening, wind-expelling, damp-eliminating, detoxifying, pain-relieving; high fever, delirium, epilepsy, deafness with qi stagnation, dreaminess forgetfulness, chest tightness, abdominal distension, anorexia, rheumatism pain, ulcer, scabies	Europe; North Asia; China (Hubei, Henan, Shanxi, Shaanxi, Chongqing)	
A. amurensis	Whole plant, rhizome	Diaphoresis, liver/kidney tonifying (whole plant); Korean medicine (paralysis, menoxenia, stomachache, pertussis (rhizome)	Russia Far East; North Korea; China (Liaoning, Jilin, Heilongjiang)	
A. anhuiensis	Rhizome	Traumatic injury, rheumatic arthritis	China (Anhui)	
A. baicalensis	Leaf	Detoxifying, vermifuge	Siberia; Korea; China (Sichuan, Gansu, Shaanxi, Qinling Mountains, Liaoning, Jilin, Heilongjiang)	
A. begoniifolia	Whole plant	Wind-expelling, damp-eliminating, detoxification, pain-relieving; rheumatism, urticaria, carbuncle sore	China (Yunnan, Guangxi, Guizhou, Sichuan, Chongqing)	
A. biflora	Bulb	Styptic, antiphlogistic; boils, burns, cuts and wounds	Kashmir Himalaya	
A. canadensis	Root, leaf	Anthelmintic, antiaphonic, antiseptic, astringent, ophthalmic, styptic; pain in the lumbar region, crossed eyes, twitches and eye poisoning, wounds, nose bleed, sore, headache and dizziness, clear the throat	Eastern and Central North America	
A. cathayensis	Rhizome	Cancer, inflammation, analgesic, convulsion	Korea; China (Shanxi, Hebei)	
A. chosenicola var. schantungensis	Root	Styptic, damp-eliminating, heat-clearing, detoxification	China (Shandong)	
A. cylindrica	Root, leaf, stem, fruit	Antiseptic; sore eyes (stem, fruit); headache, dizziness, wounds (root); burns (leaf)	Western North America	
A. davidii	Rhizome	Blood-activating, pain-relieving, subduing swelling, detoxicating; traumatic injury, arthritis pain, lumbar muscle strain; Tujia medicine (arthritis pain, intercostal neuralgia, traumatic injury, hematemesis, hemafecia)	China (Chongqing, Tibet, Yunnan, Sichuan, Guizhou, Hunan, Hubei)	
A. delavayi	Rhizome	Blood-activating, stasis-scattering, tonifying kidney	China (Yunnan, Sichuan)	
A. demissa	Root, fruit, whole plant	Whole plant: rheumatism, dysentery, help digestion, dyspepsia, gonorrhea, wind-cold-dampness arthralgia, joint yellow water; fruit: damp-clearing, mass-scattering, detoxifying, all kinds of cold, lump boil, snakebite	Himalayas; China (QTP East margin)	Tibetan medicine
A. demissa var. major	Root, fruit, whole plant	Whole plant: rheumatism, dysentery, help digestion, dyspepsia, gonorrhea, wind-cold-dampness arthralgia, joint yellow water; fruit: damp-clearing, mass-scattering, detoxifying, all kinds of cold, lump boil, snakebite	China (QTP East margin)	Tibetan medicine
A. demissa var. villosissima	Root, fruit, whole plant	Whole plant: rheumatism, dysentery, help digestion, dyspepsia, gonorrhea, wind-cold-dampness arthralgia, joint yellow water; fruit: damp-clearing, mass-scattering, detoxifying, all kinds of cold, lump boil, snakebite	Himalayas; China (QTP East margin)	Tibetan medicine

Table 1 (*continued*)

Species	Medicinal part	Therapeutic efficacy	Distribution	Note
A. dichotoma	Rhizome	Muscle-relaxing, blood-activating, heat-clearing, detoxification, traumatic injury, dysentery, rheumatoid joint pain; skin ulcer; sore throat, cough with copious phlegm, lymphnoditis	North Asia; Europe; China (Jilin, Heilongjiang)	
A. drummondii	Root, seed	Abrasions, toothed ache, rheumatism; antibacterial; sex related difficulties; melancholy (root); headache (seed)	Western North America	
A. flaccida	Rhizome	Wind-expelling, dampness-eliminating, muscle-relaxing, blood-activating; traumatic injury, arthritis pain, lumbar muscle strain	Japan; Russia Far East; China (Yunnan, Sichuan, Guizhou, Hubei, Chongqing, Hunan, Jiangxi, Zhejiang, Jiangsu, Shaanxi, Gansu)	
A. flaccida var. hofengensis	Rhizome	Wind-expelling, dampness-eliminating, muscle-relaxing, blood-activating; traumatic injury, arthritis pain, lumbar muscle strain	China (Chongqing)	
A. fulingensis	Rhizome	Wind-expelling, dampness-eliminating, muscle-relaxing, blood-activating; traumatic injury, arthritis pain, lumbar muscle strain	China (Chongqing)	
A. griffithii	Rhizome, seed	Blood-activating, pain-relieving, subduing swelling, detoxicating; traumatic injury, arthritis pain, lumbar muscle strain; Tibet medicine (stomach worms, sharp pain, snakebite, cold tumor, gonorrhoea, joint yellow water (seed)	Sikkim; Bhutan; Nepal; China (Tibet, Sichuan, Chongqing)	Tibetan medicine
A. hupehensis	Rhizome, root, stem, leaf, whole plant	Heat-clearing, diuresis, detoxification, vermifuge, stasis-scattering, detumescence; dysentery, mallnutrition and indigestion of children, malaria, acute jaundice hepatitis, ascariasis, furuncle carbuncle, scrofula, traumatic injury	China (Chongqing, Southern Shaanxi, Gansu, Zhejiang, Jiangxi, Western Hubei, Northern Guangdong, Northern Guangxi, Sichuan, Guizhou, Eastern Yunnan)	
A. hupehensis f. alba	Rhizome	Heat-clearing, diuresis, detoxification, vermifuge, stasis-scattering, detumescence; dysentery, mallnutrition and indigestion of children, malaria, acute jaundice hepatitis, ascariasis, furuncle carbuncle, scrofula, traumatic injury	China (Chongqing)	
A. hupehensis var. japonica	Rhizome	Heat-clearing, diuresis, detoxification, vermifuge, stasis-scattering, detumescence; dysentery, mallnutrition and indigestion of children, malaria, acute jaundice hepatitis, ascariasis, furuncle carbuncle, scrofula, traumatic injury	China (Chongqing)	
A. imbricata	Root, fruit, whole plant, flower, stem, leaf, seed	Whole plant: expelling wind-damp, dysentery, help digestion, gonorrhea, wind-cold-dampness arthralgia, joint yellow water; fruit: damp-expelling, mass-eliminating, detoxifying, all kinds of cold, lump boil, snakebite; stomach worms, sharp pain, snakebite, cold tumor, gonorrhoea, joint yellow water (seed); gonorrhoea, joint yellow water, hypothermia, emetic (leaf); anti-inflammatory, burn (stem, leaf and flower)	China (QTP East margin)	Tibetan medicine
A. multifida	Root, seed	Abrasions, toothed ache, rheumatism; antibacterial; sex related difficulties; melancholy (root); headache (seed)	Central and Western North America	

Table 1 (*continued*)

Species	Medicinal part	Therapeutic efficacy	Distribution	Note
A. narcissiflora	Leaf, root, seed	Abrasions, toothache, rheumatism; antibacterial; sex related difficulties; melancholy (root); headache (seed)	Europe; Asia; North America	
A. nemorosa	Various parts	Headaches, tertian agues and rheumatic gout (various parts), leprosy (leaf), bring away watery and phlegmatic humours (root), lethargy, eye inflammation, malignant and corroding ulcers (root)	UK; Europe; West Asia	
A. nikoensis	Leaf	Edible use	Japan	
A. obtusiloba	Seed, above-ground part, root, fruit	Diuresis detumescence, enriching blood, warming body, wound healing, pus drainage; antirheumatic; emetic (seed); ophthalmic; rubefacient; contusion (root); ill health, hypothermia, sore throat, chronic bronchitis, tonsillitis, hepatitis, gastric disease, dysentery, gonorrhea, arthritis pain, peripheral nerve paralysis, snakebite, stubborn dermatitis, impetigo, joint yellow water	Himalayas; China (QTP East margin)	Tibetan medicine
A. obtusiloba ssp. ovalifolia	Whole plant, aboveground part, root, fruit	Diuresis detumescence, enriching blood, warming body, wound healing, pus drainage; styptic (whole plant), ill health, hypothermia, sore throat, chronic bronchitis, tonsillitis, hepatitis, gastric disease, dysentery, gonorrhea, arthritis pain, peripheral nerve paralysis, snakebite, stubborn dermatitis, impetigo, joint yellow water	China (Taibai mountain, QTP East margin)	Tibetan medicine
A. parviflora	Root, seed	Abrasions, toothache, rheumatism; antibacterial; sex related difficulties; melancholy (root); headache (seed)	North America	
A. parviflora (*A. pulsatilla*)	Whole plant	Diaphoretic, diuretic, nervine, rubefacient; eye ailments, earache, stress, anxiety, tension, skin eruptions, rheumatism, leukorrhea, obstructed menses, bronchitis, coughs, asthma	UK; Europe	
A. quinquefolia	Root	Rubefacient; rheumatism, gout, fever; vesicant, corns	Eastern North America	
A. raddeana	Rhizome	Mongolian medicine: rheumatism, low back and leg pain, phlebitis; subduing inflammation; arthralgia, chill cold, cough with copious phlegm, joint pain	China (Northeastern Shandong, Liaoning, Jilin, Heilongjiang); Korea; Russia Far East	Chinese Pharmacopoeia
A. reflexa	Rhizome	Open the orifices with aroma, wind-expelling, dampness-eliminating, appetite-stimulating; high fever, delirium, epilepsy, deafness with qi stagnation, dreaminess forgetfulness, chest tightness, abdominal distension, anorexia, rheumatism pain, ulcer, scabies	North Korea; Siberia; Eastern Europe; China (Shaanxi, Eastern Jilin, Taibai mountain)	
A. rivularis	Rhizome, leaf, seed; aboveground part, root, fruit	Heat-clearing, detoxification, blood-activating, muscle-relaxing, swell-dispersing, pain-relieving; diuresis detumescence, enriching blood, warming body, wound healing, pus drainage; mumps, scrofula, carbuncle, malaria, cough, jaundice, arthritis pain, traumatic injury, stomachache, toothache; ill health, hypothermia, sore throat, chronic bronchitis, tonsillitis, hepatitis, gastric disease, dysentery, gonorrhea, arthritis pain, peripheral nerve paralysis, snakebite, stubborn dermatitis, impetigo, joint yellow water	China (Chongqing, Tibet, QTP East margin); Himalayas; Sri Lanka	Tibetan medicine

Table 1 (*continued*)

Species	Medicinal part	Therapeutic efficacy	Distribution	Note
A. rivularis var. flore-minore	Rhizome	Heat-clearing, detoxification, blood-activating, muscle-relaxing, swell-dispersing, pain-relieving; sore throat, mumps, scrofula, carbuncle, malaria, cough, jaundice, arthritis pain, traumatic injury, stomachache, toothache	China (Chongqing)	
A. rockii var. pilocarpa	Rhizome	Wind-expelling, dampness-removing, muscle-relaxing, blood-activating; arthritis pain, traumatic injury	China (Chongqing)	
A. rupicola	Seed	Stomach worms, sharp pain, snakebite, cold tumor, gonorrhoea, joint yellow water	China (Northwestern Yunnan, Western Sichuan, Southeastern and Southern Tibet); Bhutan; Nepal; Northern India	Tibetan medicine
A. silvestris	Rhizome	Relieving oppression and masses, pus drainage, rot-eliminating, insecticide	Europe; Asia; China (Liaoning, Hebei, Heilongjiang, Jilin, Xinjiang, Inner Mongolia)	Mongolian medicine "Xiriwusu"
A. stolonifera	Leaf, stem	Edible use	China; Japan	
A. taipaiensis	Rhizome	Cancer	China (Taibai mountain)	
A. tetrasepala	Seed	Stomach worms, sharp pain, snakebite, cold tumor, gonorrhoea, joint yellow water	China (Southern Tibet); Kashmir; Afghanistan	Tibetan medicine
A. tibetica	Seed	Stomach worms, sharp pain, snakebite, cold tumor, gonorrhoea, joint yellow water	China (Tibet)	Tibetan medicine
A. tomentosa	Rhizome	Dissipating phlegm stasis, relieving dyspepsia, detoxification, vermifuge; eparsalgia cough, traumatic injury, mallnutrition and indigestion of children, malaria, dysentery, sore furuncle carbuncle, stubborn dermatitis	China (Chongqing, Sichuan, Gansu, Henan, Shanxi)	
A. trullifolia	Root, flower	Muscle-relaxing, blood-activating, antitussive; chronic bronchitis, peripheral nerve paralysis, neuralgia, tendon complex pain	China (QTP East margin, Southern Tibet); Sikkim; Bhutan	Tibetan medicine
A. trullifolia var. linearis	Root, flower	Muscle-relaxing, blood-activating, antitussive; chronic bronchitis, peripheral nerve paralysis, neuralgia, tendon complex pain	China (QTP East margin)	Tibetan medicine
A. tuberosa		Anxiolytic	Southwest America	
A. virginiana	Root, seed	Astringent; emetic; expectorant; TB, whooping cough, diarrhea; boils	Central and Eastern North America	
A. vitifolia	Root, leaf, rhizome	Traumatic injury, rheumatic arthralgia, enteritis, dysentery, ascariasis (rhizome); antirheumatic and vermifuge, dysentery, relieve tooth pain and headache, scabies (root), head lice (leaf)	Europe; Himalayas	

3.1. Saponin

Saponins are abundant in Ranunculaceae, especially in *Clematis*, *Pulsatilla*, *Anemone*, and Cimicifugeae[1,19–21], which usually exert anticancer activity *via* cell cycle arrest and apoptosis induction. The aglycones of *Clematis* pentacyclic triterpene saponins mainly belong to oleanolic type (A), olean-3β, 28-diol type (B), hederagenin type (C) or hederagenin-11,13-dien type (D), where types A and C are predominant[20]. In *Anemone*, A type (Fig. 2 and Supplementary Table S1) is predominant, and ursane-type triterpenoids (B type), lupane-type triterpenoids (C type), and cycloartane-type tetracyclic triterpenoids (D type) are also present (Fig. 3 and Supplementary Table S2).

3.2. Essential oil, volatile compounds and others

Nineteen essential oil compounds were identified from the roots of *A. rivularis*, representing 96.1% of the total oil[22] (Figs. 4 and 5, and Supplementary Table S3). The major constituents were acetophenone (55.9%), 3-ethyl-2-methyl-hexane (16.2%), 5,6-dimethyl-decane (5.9%) and 4,5-diethyl-octane (4.4%).

The dominant benzenoid compounds in *A. sylvestris* anther were 2-phenylethanol and phenylacetaldehyde[23]. Other abundant compound classes in *A. sylvestris* were fatty acid derivatives (41.8%), especially pentadecane and nonanal, and sesquiterpenoids (8.0%), *e.g.*, *(E,E)*-α-farnesene. Relatively low amounts of the repellent protoanemonin were found in *A. sylvestris*.

Coumarins, flavonoids, lactones, lignans, steroids, phenolic compounds, and other compounds are also detected (Figs. 4 and 5, and Supplementary Table S3).

4. Bioactivities

4.1. Anticancer activity: cell death pathways and anticancer targets

The genus *Anemone*, evolutionarily closely related to *Pulsatilla*, is also rich in therapeutic saponins[1]. Raddeanin A, a pentacyclic triterpene saponin from *A. raddeana* (Liang Tou Jian in TCM), inhibits proliferation and induces apoptosis of multiple cancer cell lines[7,24,25]. Raddeanin A increased BAX expression, reduced BCL-2, BCL-xL and survivin expression, and significantly activated caspase-3, caspase-8, caspase-9 and poly-ADP ribose polymerase (PARP)[25]. Saponins B, 1, and 6 of *A. taipaiensis* exhibit significant anticancer activity against human leukemia, glioblastoma multiforme (GBM), and HCC[26–31]. Saponin 1 caused characteristic apoptotic morphological changes in GBM cells[26], which was confirmed by DNA ladder electrophoresis and flow cytometry. Saponon 1 also caused a time-dependent decrease in the expression and nuclear location of NF-κB. The expression of inhibitors of apoptosis (IAP) family members, *e.g.*, survivin and XIAP, was significantly decreased by saponin 1. Moreover, saponin 1 caused a decrease in the BCL-2/BAX ratio and initiated apoptosis by activating caspase-9 and caspase-3 in the GBM cell lines. Thus, saponin 1 inhibits cell growth of GBM cells at least partially by inducing apoptosis and inhibiting survival signaling mediated by NF-κB.

Saponin B blocked the cell cycle at the S phase[31]. Saponin B induced chromatin condensation of U87MG GBM cells and led to the formation of apoptotic bodies. Annexin V/PI assay suggested that phosphatidylserine (PS) externalization was apparent at higher drug concentrations. Saponins B and 6 activated the receptor-mediated pathway of apoptosis *via* the activation of FAS-l[28].

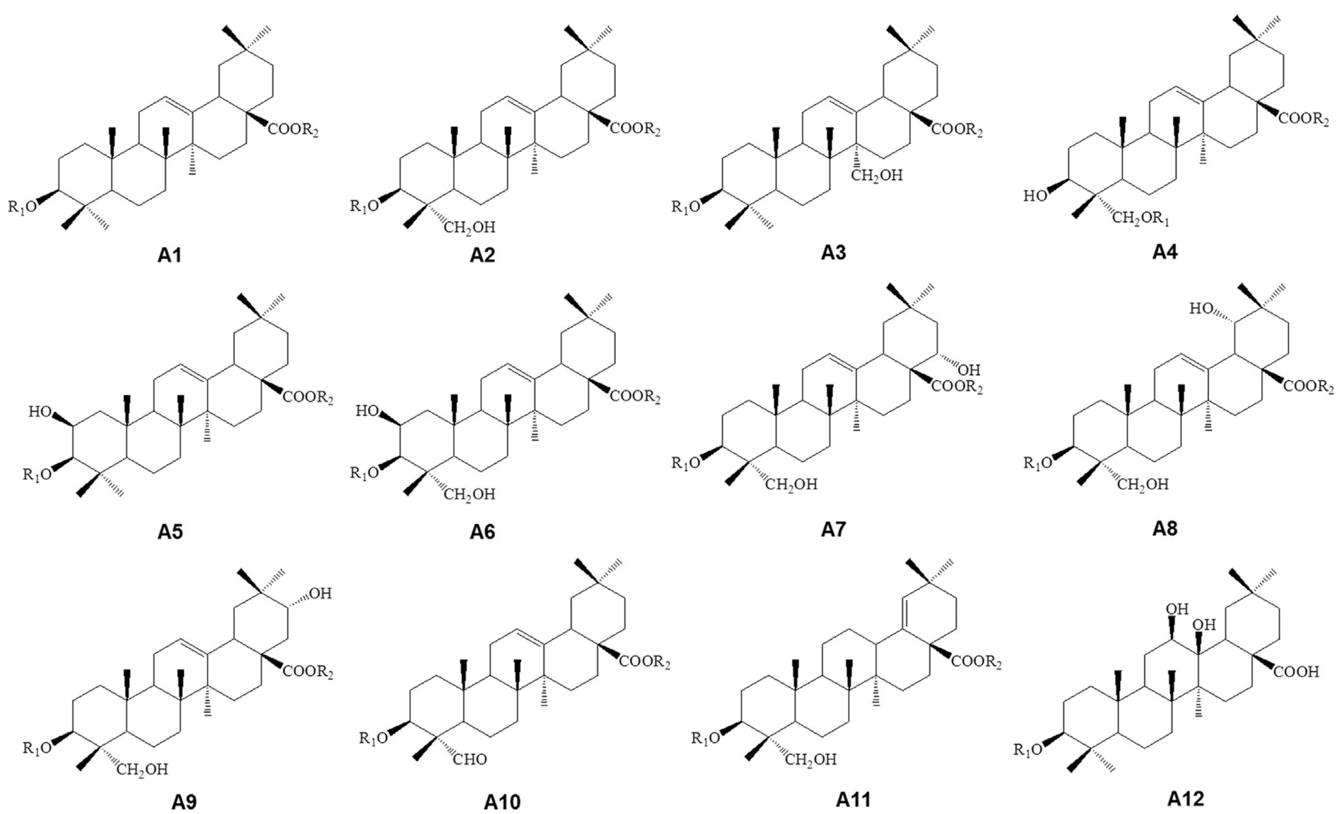

Figure 2 Basic skeletons of oleanane-type triterpenoids (A type) from *Anemone* species.

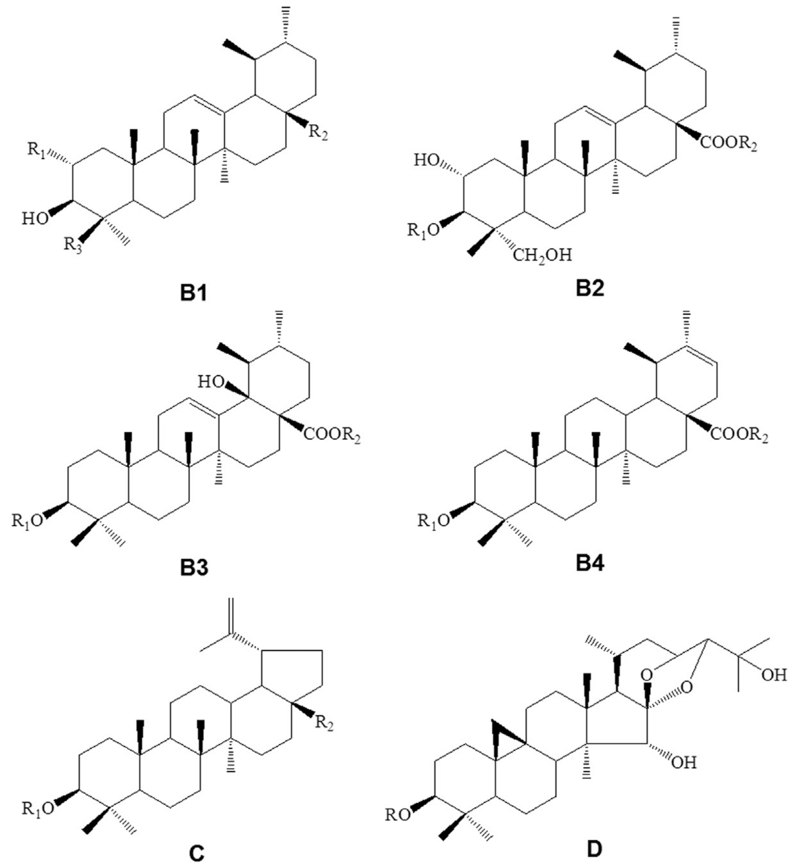

Figure 3 Basic skeletons of other type triterpenoids (B and C types) from *Anemone* species.

These saponins increased the BAX and caspase-3 ratio and decreased the protein expression of BCL-2.

Triterpenoid saponins of *A. flaccida* induce apoptosis in human BEL-7402, HepG2 hepatoma cell lines, and lipopolysaccharide (LPS) stimulated HeLa cells *via* COX-2/PGE_2 pathway[32]. Flaccidoside II, one of the triterpenoid saponins of *A. flaccida*, induced apoptosis by downregulating heme oxygenase (HO)-1 *via* extracellular signal-regulated kinase (ERK)-1/2 and p38 mitogen-activated protein kinase (MAPK) pathways[33].

Raddeanin A significantly inhibited human umbilical vein endothelial cell (HUVEC) proliferation, motility, migration, and tube formation[7]. Raddeanin A dramatically reduced angiogenesis in chick embryo chorioallantoic membrane, restrained the trunk angiogenesis in zebrafish, and suppressed angiogenesis and growth of human HCT-15 colorectal cancer xenograft in mice. Raddeanin A suppressed VEGF-induced phosphorylation of VEGFR2 and its downstream protein kinases including PLCγ1, JAK2, FAK, Src, and Akt. In a molecular docking simulation, Raddeanin A formed hydrogen bonds and hydrophobic interactions within the ATP-binding pocket of VEGFR2 kinase domain.

Raddeanin A significantly inhibited the invasion, migration and adhesion of the BGC-823 human gastric cancer cells[25]. Raddeanin A could up-regulate the expression of reversion inducing cysteine rich protein with Kazal motifs (RECK) and E-cadherin, and down-regulate the expression of matrix metalloproteinase-2 (MMP-2), MMP-9, MMP-14 and RhoC.

In a screen of 70 species of medicinal plants, the aqueous extract of *A. altaica* (AAE) had the best ability to suppress the viability of HOS and U2OS human osteosarcoma cells in a concentration-dependent manner[34]. AAE suppressed the growth of HOS and U2OS through the intrinsic apoptotic pathway, but it had no significant influence on human osteoblast hFOB cells. The high mRNA levels of apoptosis-related factors (PPP1R15A, SQSTM1, HSPA1B, and DDIT4) and cellular proliferation markers (SKA2 and BUB1B) were significantly altered by the AAE treatment. AAE could up-regulate the expression of a cluster of genes, especially those in the apoptosis-related factor family and caspase family.

4.2. *Immunomodulatory activity*

ARS, the saponins extracted from the rhizome of *A. raddeana*, showed a slight hemolytic effect and enhanced significantly the specific antibody and cellular response against ovalbumin in mice[35]. A neutral polysaccharide fraction (ARP) from the rhizome of *A. raddeana* extraordinarily promotes splenocyte proliferation, NK cell and CTL activity, as well as serum IL-2 and TNF-α production in HCC-bearing mice[2]. ARP had no toxicity to body weight, liver, and kidney. Moreover, it could reverse the hematological parameters induced by 5-fluorouracil to near normal.

4.3. *Anti-inflammatory and antioxidant activities*

Ranunculaceae tribes and genera, such as *Ranunculus*, Anemoneae, *Cimicifuga*, *Helleborus*, *Nigella*, Delphinieae, *Semiaquilegia*, *Coptis*, and *Hydrastis*, are rich in both anti-inflammatory and

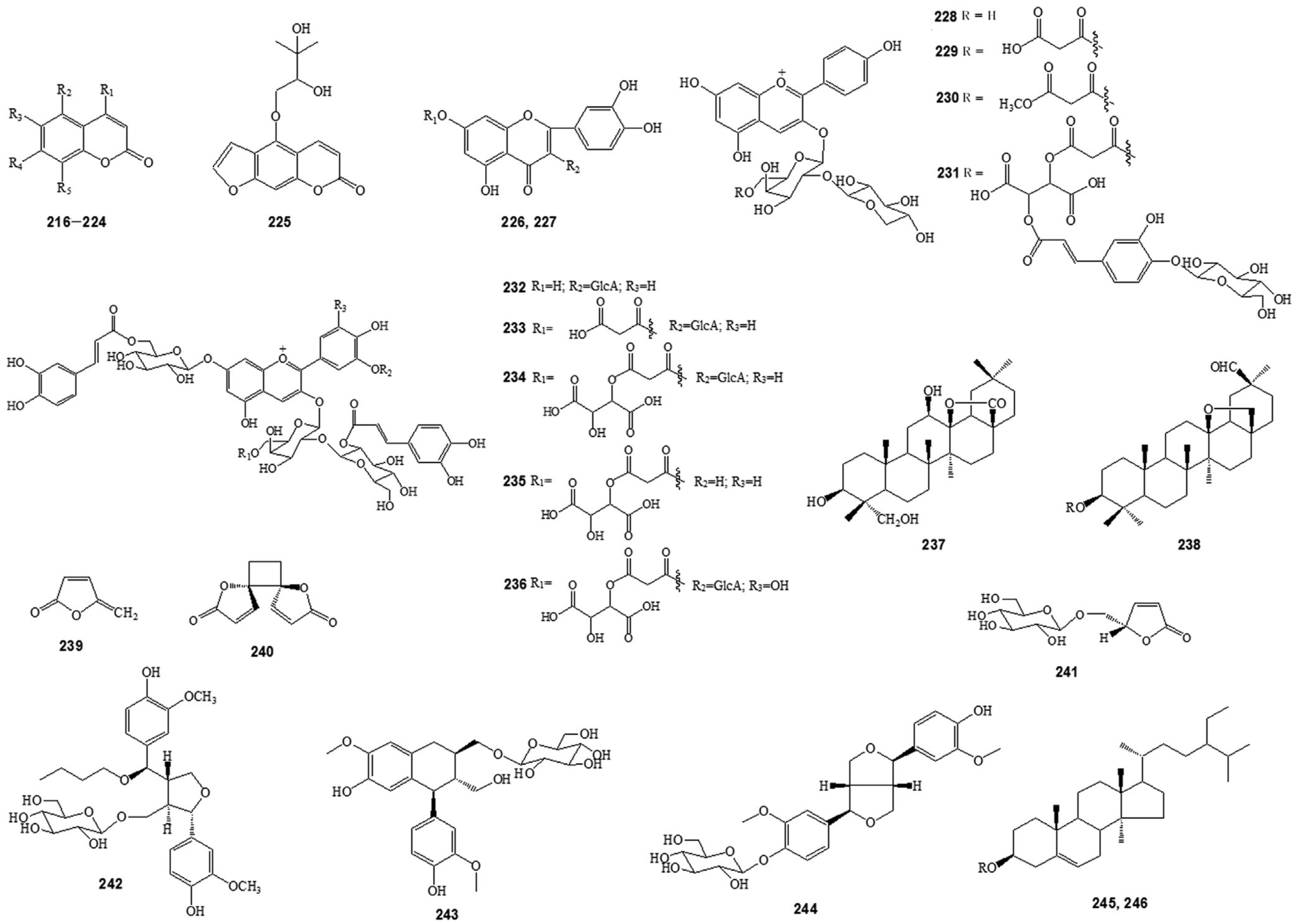

Figure 4 Constituents isolated from *Anemone* species (See substituent groups R_n in Supplementary Table S3).

anticancer phytometabolites[1,19]. Anemonin and ranunculin, the potent anti-inflammatory and anticancer compounds, are abundant in tribes Ranunculeae and Anemoneae[1,36].

A. flaccida (Di Wu in Chinese) crude triterpenoid saponins (AFSs) inhibited redness and swelling of the right hind paw in the type II collagen-induced arthritis (CIA) model in rats[4]. The inflammatory responses were reduced by AFS treatment. The serum pro-inflammatory cytokines TNF-α and IL-6 were decreased in AFS-treated CIA rats at the dose of 200 and 400 mg/kg/day. AFS and its main compounds, including hederasaponin B, flaccidoside II, and hemsgiganoside B, significantly inhibited TNF-α and IL-6 production in LPS-treated RAW264.7 cells, respectively.

Osteoclasts are bone-specialized multinucleated cells and are responsible for bone-destructive diseases, such as rheumatoid arthritis and osteoporosis. In RAW264.7 cells and CIA rats, the total saponin (TS) of *A. flaccida* concentration-dependently inhibited receptor activator of NF-κB ligand (RANKL)-induced osteoclast formation and bone marrow-derived macrophages (BMMs), as well as decreased extent of actin ring formation and lacunar resorption[6,37]. The RANKL-stimulated expression of osteoclast-related transcription factors was also diminished by TS, while the expression of osteoprotegerin (OPG), at both mRNA and protein levels increased, and the ratio of RANKL to OPG in inflamed joints and sera of CIA rats decreased[37]. TS blocked the RANKL-triggered TRAF6 expression, phosphorylation of MAPKs and IκB-α, and inhibited NF-κB p65 DNA binding activity. TS almost abrogated the nuclear factor of activated T cells (NFATc1) and c-Fos expression. TS suppresses RANKL-induced osteoclast differentiation and inflammatory bone loss *via* the down-regulation of TRAF6 level, suppression of c-jun N-terminal kinase (JNK) and p38 MAPK and NF-κB activation, and subsequently decreased expression of c-Fos and NFATc1. The triterpenoid saponin W3 of *A. flaccida* had similar effects[38]. Therefore, TS and the saponins thereof may be useful for lytic bone diseases and further *in vivo* studies and clinical trials are warranted.

Two coumarins of *A. raddeana* had inhibitory effect against human leukocyte elastase[39]. 3-Acetyloleanolic acid (AOA), oleanolic acid (OA), raddeanoside 12 (Rd12) and Rd13, isolated from *A. raddeana*, suppressed the superoxide generation induced by *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) in a concentration-dependent manner[40]. Eleutheroside K (EK) and Rd10 significantly enhanced fMLP-induced superoxide generation in low concentration (0.5–0.75 μmol/L), while these compounds more efficiently suppressed superoxide generation than the other four compounds in other concentrations. Rd12 dose-dependently inhibited fMLP-induced tyrosyl phosphorylation of 123.0, 79.4, 60.3, 56.2 and 50.1 kDa proteins in human neutrophil, while Rd10 and EK enhanced the tyrosyl phosphorylation of these proteins at a low concentration range.

Superoxide generation induced by fMLP was significantly suppressed by betulin and lupeol, extracted from the roots of

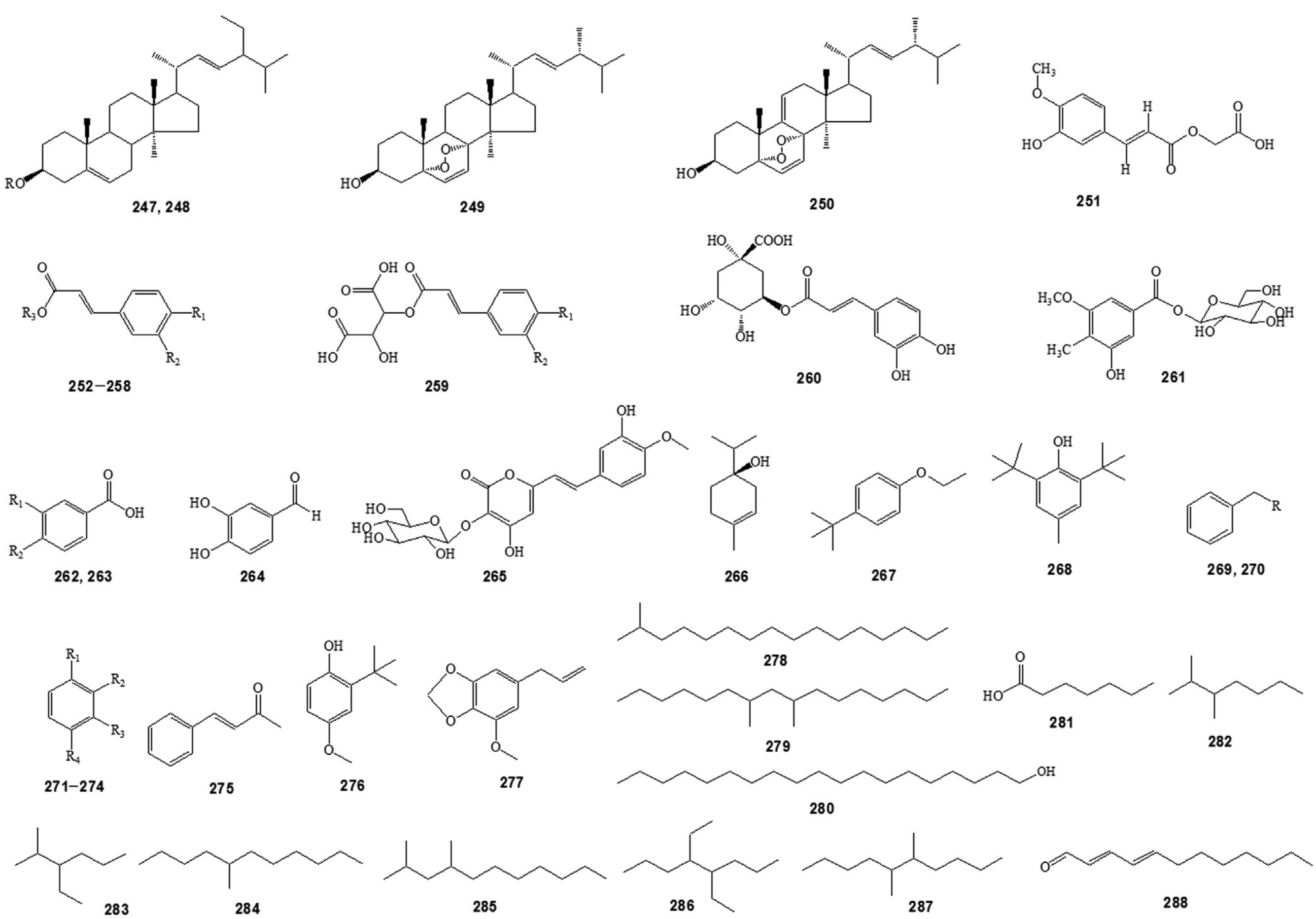

Figure 5 Other constituents isolated from *Anemone* species (See substituent groups R_n in Supplementary Table S3).

A. raddeana, depending on the concentration of the triterpenoids[41]. The suppressive effect of betulinic acid was low. The phorbol 12-myristate 13-acetate (PMA)-induced superoxide generation was suppressed by betulin in a concentration-dependent manner, but not by lupeol and betulinic acid. However, superoxide generation induced by arachidonic acid (AA) was suppressed by lupeol, while betulin and betulinic acid weakly enhanced AA-induced superoxide generation. Lupeol and betulin suppressed tyrosyl phosphorylation of a 45.0-kDa protein in fMLP-treated human neutrophils, but betulinic acid did not. Lupeol, betulin and betulinic acid showed no hemolytic effect even at the concentration of 500 μmol/L.

Five oleanolic acid triterpenoid saponins (OTSs), isolated from the rhizome of *A. raddeana*, suppressed fMLP-induced superoxide generation in a concentration-dependent manner[42]. OTS-1, 2 and 4 suppressed PMA- and AA-induced superoxide generation in a concentration-dependent manner, but OTS-3 and -5 showed no effect. fMLP- and PMA-induced tyrosyl or serine/threonine phosphorylation, and fMLP-, PMA- and AA-induced translocation of p67 (phox), p47 (phox) and Rac to plasma membrane were in parallel with the suppression of the stimulus-induced superoxide generation.

4.4. Antimicrobial activity

The antioxidant essential oil, obtained from the roots of *A. rivularis*, had antibacterial activity[22]. The inhibition zones at 100 μg/disc and minimum inhibitory concentration (MIC) values for four bacterial strains were in the range of 11.0–20.0 mm and 125–250 μg/mL, respectively.

5. Taxonomy and pharmacophylogeny

The distribution of anticancer compounds within Ranunculaceae is not random but phylogeny-related[43–45]. For instance, *Ranunculus*, *Clematis*, *Pulsatilla*, *Anemone*, and *Nigella* are rich in pentacyclic triterpene saponins. *Pulsatilla*, *Anemone*, and *Clematis* belong to the tribe Anemoneae, and *Pulsatilla* is evolutionarily more close to *Anemone* than to *Clematis*[1]. *Clematis* is closer to *Naravelia* and *Anemoclema* than to *Anemone* and *Pulsatilla. Hepatica* is basal to all other genera of Anemoneae[46]. The sister group relationship between Ranunculeae and Anemoneae is revealed by two independent groups[46,47].

Previous phylogenies based on molecular data indicated that segregate genera from both the Northern and Southern Hemispheres (*Hepatica*, *Pulsatilla*, *Knowltonia*, *Oreithales*, and *Barneoudia*) are embedded within *Anemone* and should be subsumed within the genus. Based on a new phylogeny that substantially increases the sampling of the austral anemones (especially from Africa), Hoot et al.[48] analyzed combined sequence data (chloroplast *atpB-rbcL* spacer and nuclear ITS regions) for 55 species of *Anemone*, using Bayesian inference, maximum likelihood (ML), and maximum parsimony. The segregate genera, *Oreithales* and *Barneoudia*, nest within *Anemone* and are included in a well-supported clade (subgenus *Anemone*, section *Pulsatilloides*)

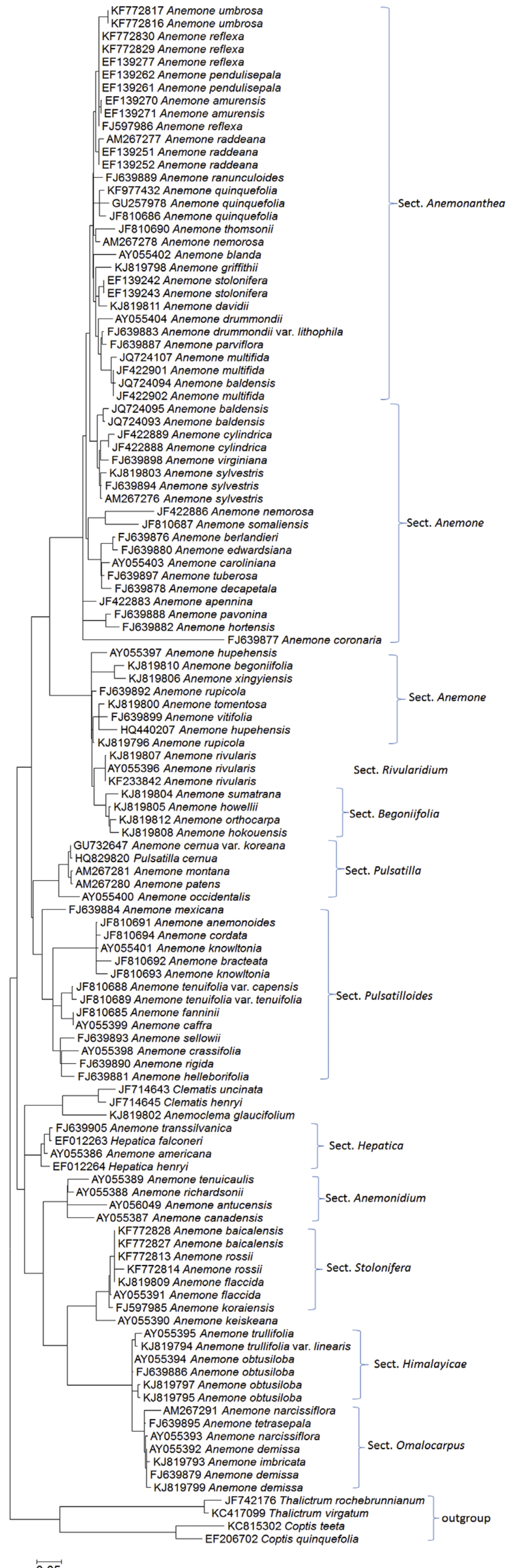

Figure 6 Phylogenetic relationship of *Anemone* ITSs inferred by ML (maximum likelihood) method. Scale bar represents 0.05 substitutions per site.

consisting largely of Southern Hemisphere species. The Mexican *A. mexicana* is sister to all remaining members of section *Pulsatilloides* (Supplementary Fig. S1), which consists of two clades: a poorly supported South American and Tasmanian clade (*A. sellowii*, *A. helleborifolia*, *A. rigida*, *Barneoudia* and *Oreithales* species, and *A. crassifolia*) and a highly supported Southern African clade including 9 species of *Knowltonia* and 8 species of *Anemone. A. antucensis* (Chile, Argentina) falls in a separate clade (subgenus and section *Anemonidium*) that is sister to *A. tenuicaulis* (New Zealand). *A. thomsonii* (Eastern Africa) and *A. somaliensis* (Somalia) are in a clade (subgenus and section *Anemone*) composed largely of Northern Hemisphere species. *A. somaliensis* is further associated with other Mediterranean tuberous anemones in subsection and series *Anemone* (*A. coronaria*, *A. hortensis*, and *A. pavonina*). The topology of both sections *Pulsatilloides* and *Anemonidium* suggests that anemones originated in the Northern Hemisphere and subsequently spread to the Southern Hemisphere, a pattern that is shared with other members of Ranunculaceae.

The taxonomic relationship of many Chinese species is elusive, we thus retrieved ITS sequences of more *Anemone* species from the NCBI GenBank and reconstructed their phylogenetic relationship (Fig. 6). *Anemone umbrosa*, *A. reflexa*, *A. amurensis*, *A. raddeana*, *A. griffithii*, *A. stolonifera*, and *A. davidii*, belonging to the section *Anemonanthea*, cluster on the top of the phylogenetic tree, which is congruent to the morphological classification. Some non-Chinese species, *e.g.*, *Anemone virginiana*, *A. sylvestris*, *A. somaliensis*, and *A. tuberosa*, belonging to the section *Anemone*, are below the *Anemonanthea* species. However, some Chinese species of the section *Anemone*, *e.g.*, *Anemone hupehensis*, *A. begoniifolia*, *A. xingyiensis*, and *A. rupicola*, are closer to *A. rivularis* (Sect. *Rivularidium*), *A. orthocarpa*, and *A. hokouensis* (Sect. *Begoniifolia*). All above taxa belong to the morphological subgenus *Anemone*. The sections *Pulsatilla* and *Pulsatilloides*, belonging to the subgenus *Anemone*, have no Chinese taxa. On the other hand, five sections, belonging to the subgenus *Anemonidium*, form another major clade. The section *Himalayicae* is closer to the section *Omalocarpus* than to the section *Stolonifera*. The non-Chinese sections *Hepatica* and *Anemonidium* are basal to these sections. The sections *Himalayicae* and *Omalocarpus*, evolutionarily younger than other sections, have some important Tibetan medicinal plants, *e.g.*, *Anemone trullifolia*, *A. obtusiloba*, *A. demissa*, and *A. imbricata*. These two groups are still in the process of rapid radiation, corresponding to the extensive uplift of Qinghai–Tibet Plateau (QTP) during Quaternary[45]. The numerous morphologically and phytochemically distinct species should be investigated in detail to facilitate the sustainable development of *Anemone*-based clinical therapy.

6. Transcriptomics, proteomics and metabolomics

Transcriptome sequencing and proteomic techniques were combined to comprehensively analyze the triterpenoid saponin biosynthetic pathway in *Anemone flaccida*[49]. A total of 126 putative cytochromes P450 (CYP) and 32 UDP glycosyltransferases were selected from 46, 962 unigenes as candidates for triterpenoid saponin modifiers. Four CYPs were annotated as genes of CYP716A subfamily, the key enzyme in the oleanane-type saponin biosynthetic pathway. Based on RNA-Seq, iTRAQ proteome analysis, and quantitative RT-PCR verification, the expression level of genes and proteins committed to triterpenoids biosynthesis in the leaf and the rhizome could be compared. *De novo* transcriptome and proteome profiling are powerful in the discovery of candidate genes, which are related to the biosynthesis of phytometabolites in a non-model plant. The transcriptome of *A. coronaria*[50], following infection with rust, is available, allowing for comparative transcriptomic studies. Twenty taxonomically related benzylisoquinoline-alkaloid-producing plants, belonging to Ranunculaceae and closely related families, were subjected to the transcriptome sequencing and analysis[51]. These essential data resources can be used to isolate and discover functional homologues and novel catalysts within the metabolism of medicinal compounds. Orthologs could be extracted for transcriptome-based phylogeny reconstruction[52,53].

Metabolomics studies provide imperative insight into the primary biochemical networks behind specialized metabolism and contribute key resources for metabolic engineering, gene discovery, and elucidation of regulatory mechanisms[54]. Future comprehensive and thorough metabolomics investigations of *Anemone* species are warranted.

7. Conclusion and future prospects

Are triterpenoid saponins of *Anemone* plants epiphany molecules for cancer patients? Not yet. Much more *in vivo* evidence has to be collated in experimental animals and humans, while at present there is a lack of pharmacokinetic and pharmacodynamic data on *Anemone* anticancer compounds. The structure–activity relationships should be investigated for understanding the molecular mechanisms and rational drug design. Some new *Anemone* taxa have been identified in Southwest China[55–57], the biodiversity center of Ranunculaceae plants. A majority of *Anemone* species have not been probed with respect to their unique biosynthetic pathways and chemodiversity. A trade-off should be recognized between the conservation of endangered *Anemone* species and the utilization of *Anemone* pharmaceutical resources. The advent of the genomic era has provided important and surprising insights into the deducted genetic composition of *Anemone* species. Various innovative "omics" platforms would be of great help in deciphering biosynthetic pathways of *Anemone* phytometabolites, which will provide a solid foundation for future synthetic biology manipulations and also help protect *Anemone* medicinal resources for sustainable utilization.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2016.12.001.

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