Review Article

Genus Caulophyllum: An Overview of Chemistry and Bioactivity

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Recently, some promising advances have been achieved in understanding the chemistry, pharmacology, and action mechanisms of constituents from genus Caulophyllum. Despite this, there is to date no systematic review of those of genus Caulophyllum. This review covers naturally occurring alkaloids and saponins and those resulting from synthetic novel taspine derivatives. The paper further discussed several aspects of this genus, including pharmacological properties, mechanisms of action, pharmacokinetics, and cell membrane chromatography for activity screening. The aim of this paper is to provide a point of reference for pharmaceutical researchers to develop new drugs from constituents of Caulophyllum plants.

1. Introduction

Caulophyllum is a small genus of perennial herbs in the family Berberidaceae. The genus Caulophyllum is well known for its diversity and pharmacological uses in traditional medicine system since ancient times. All species in this genus are very similar [1]. C. robustum is native to eastern Asia, especially in China, while C. thalictroides and C. giganteum are native to eastern North America. It is worth noting that nearly all phytochemical and pharmacological studies on this genus are focused on C. thalictroides and C. robustum due to their important medical functions [2].

The roots and rhizomes of C. thalictroides (L.) Michx. (blue cohosh) have been used traditionally by Native Americans for medicinal purposes [3]. The primary function of blue cohosh in many native communities of North America was to induce childbirth, ease the pain of labor, rectify delayed or irregular menstruation, and alleviate heavy bleeding and pain during menstruation [4]. Between 1882 and 1905, blue cohosh was listed in the United States Pharmacopoeia as a labor inducer [5] and sold as an herbal supplement that can aid in childbirth. Dietary supplements of blue cohosh are readily available throughout the USA over-the-counter and from Internet suppliers [6]. There is considerable concern about the safety of blue cohosh with reports of newborn babies having heart attacks or strokes after the maternal consumption of blue cohosh to induce labor [7–9]. There is a heated discussion about using blue cohosh as dietary supplements for women [2].

C. robustum Maxim is well-known in Hong Mao Qi in Chinese, which grows widely throughout north-east, north-west, and south-west China. Its roots and rhizomes have been used as folk medicine to treat external injuries, irregular-menses, and stomach-ache due to its strong and wide biological activities [10]. Modern pharmacological studies have demonstrated that alkaloids and triterpence saponins are responsible for its major biological function as an anti-inflammatory [11], analgesic [12], antioxidant [13], antibacterial [11], antiaethylcholinesterase [14], and antitumor [15, 16]. Taspine, a lead compound in anticancer agent development [17, 18], was firstly screened to possess obvious effect on tumor angiogenesis and human epidermal growth factor receptor by using cell membrane chromatography from the C. robustum [19].

So it is very necessary to deeply explore Caulophyllum plants. In the past decades, some promising advances have been achieved in understanding the chemistry, pharmacology, and action mechanisms of constituents from genus...
Caulophyllum. From the opinion of safety of using dietary supplements of blue cohosh, a review dealing with quantitative methods of primary constituents of blue cohosh in dietary supplements has been published [2]. However, to date, there is no systematic review of chemistry, pharmacology, and action mechanisms of constituents from genus Caulophyllum.

In this review, the different structures of the alkaloids and saponins in genus Caulophyllum are described, including naturally occurring constituents and synthetic taspine derivatives. The present review highlighted the chemistry and pharmacological diversity and mechanism of action. The aim of this paper is to provide a point of reference on Caulophyllum plants for pharmaceutical researchers. Furthermore, various perspectives and existing problems for this genus are offered for consideration.

2. Phytochemistry

Phytochemical research carried out on genus Caulophyllum led to the isolation of alkaloids and triterpene saponins and a few other classes of secondary metabolites. A comprehensive summary of structures and isolation methods of metabolites classified by structural types was given in present review. Scheme 1 summarizes the procedures for crude isolation of alkaloids and triterpene saponins from genus Caulophyllum. The roots and rhizomes of Caulophyllum plants are extracted with methanol or 70% ethanol by maceration [13, 20] or reflux [21], and the combined extracts are concentrated in vacuo to dryness. Then two schemes are available for acquiring the alkaloid and saponin fractions, namely, liquid-liquid partition and liquid-solid column chromatography methods [21]. Liquid-liquid partition is commonly performed for crude isolation. In most cases, the residue is suspended in 5% or 0.1 N HCl in water and then partitioned with EtOAc or CHCl₃ to remove neutral constituents. The aqueous layer was then removed, NH₄OH was added to make it basic (pH 9), and the whole was extracted with EtOAc or CHCl₃. The EtOAc or CHCl₃ soluble part was evaporated to obtain the total alkaloidal fraction. Moreover, total alkaloidal fraction was able to further liquid-liquid partition to afford weak
base (Fr. 1), nonphenolic alkaloids (Fr. 2), and phenolic alkaloids (Fr. 3) [13]. The H₂O layer was neutralized with 5% HCl and extracted with n-butanol. The combined organic layers were evaporated to obtain total saponin fraction [20]. Column chromatography is also a popular method to enrich total alkaloids and saponins from Caulophyllum plants by choosing optimal macroporous or (and) ion exchange resins [13, 21, 22].

2.1. Alkaloids. With respect to alkaloid aspects of this genus, 22 molecules have been isolated and identified from genus Caulophyllum. Alkaloid compounds are very important bioactive constituents in genus Caulophyllum. Their chemical structures and sources can be seen in Figure 1 and Table 1. These compounds can be divided into several kinds of structural types. magnoflorine (1), taspine (2), and boldine (3) are contributed to aporphine alkaloids. Aporphine alkaloids have been shown to possess anticancer activity and there is evidence that this activity is exerted through induction of apoptosis, inhibiting cell proliferation and inhibiting DNA topoisomerase [23, 24]. Magnoflorine (I), a quaternary ammonium base, is isolated and detected with the biggest amounts among all the alkaloids isolated from genus Caulophyllum. I was also isolated from the n-butanol fraction of blue cohosh due to its strong water-solubility, but it was not active in the rat embryo culture [25]. The molecular structure of 2 is characterized by high symmetry. 4–12 are typical quinolizidine alkaloids.

**Figure 1:** Chemical structures of alkaloids (1–22) from genus Caulophyllum.
Quinolizidine alkaloids have been reported to possess the obvious nematicidal activity [26].

In October 1999, a novel alkaloid, thalictroidine (13) with piperidine-acetophenone conjugate, was isolated from the rhizomes of C. thalictroides using an in vitro rat embryo culture method. 13 was not teratogenic in the rat embryo culture at tested concentrations [25]. After nine years, 13 was isolated again from C. thalictroides, together with 14–16 [20]. 13–15, piperidine-acetophenone conjugates, are rare in the plant kingdom. 16 was only reported from Boehmeria genus [27] and is another example of such a type of compound from natural sources.

In April 2009, a distinct class of alkaloid, fluoronene alkaloid (caulophine, 17), was firstly reported from the radix of C. robustum using cell membrane chromatography as the screening method. 17 was identified as 3-((2-(dimethylamino)ethyl)-4,5-dihydroxy-1,6-dimethoxy-9H-fluoren-9-one based on physicochemical and spectroscopic analyses. 17 possessed antomyocardial ischemia activity by rat experiments. It is worth mentioning that a preparative high performance liquid chromatography method was developed for isolation, purification, and enrichment of caulophine (17) [28]. As follows, another four fluoronene alkaloids, caulophyllines A–D (18–21), and one dihydroazafluoranthen alkaloid, caulophylline E (22), were isolated from the roots of C. robustum.

Fluoronene alkaloid is a newly discovered alkaloid skeleton in natural products. 17–21, five new fluoronene alkaloids, were isolated from the same plant, suggesting that fluoronene type alkaloid is another kind of metabolites that existed in this genus Caulophyllum. 22 is a novel and rare naturally occurred dihydroazafluoranthen alkaloid, there are no reports about dihydroazafluoranthen alkaloid isolated from natural products except its novel core skeleton first isolated from coal tar [24]. 22 has the isoquinoline fragment, which is possible to be the conceivable precursor of different substituted fluoronene alkaloids. A hypothetical biosynthetic pathway for 20 was proposed starting from 22, which undergoes a sequential nitrogen-related double bond reduction, oxidation, ring-opening, N-methylation, and demethoxy process [13].

### 2.2. Triterpene Saponins

Caulophyllum triterpenes generally constitute the main class of secondary metabolites in the genus Caulophyllum amounting to up to 7.46% of the total dry weight in root and rhizome [29]. Until now, 32 caulophyllsaponins were isolated and identified by chemical and detailed spectroscopic analysis (Table 2). These saponins generally bear one (monodesmosidic) or two (bidesmosidic) carbohydrate chains that are directly attached to the hydroxyl groups in position C-3 for monodesmosidic saponins and to positions C-3 and C-28 in the case of the bidesmosidic saponins.

#### Table 1: Chemical structures of alkaloids (1–22) from genus Caulophyllum.

| No. | Compounds | Formula | Sources | References |
|-----|-----------|---------|---------|------------|
| 1   | Magnoflorine | C_{20}H_{24}N_{2}O_{4} | Cr, Ct | [85, 86] |
| 2   | Taspine | C_{20}H_{24}N_{2}O_{4} | Cr, Ct | [25, 86] |
| 3   | Boldine | C_{20}H_{24}N_{2}O_{4} | Cr | [86] |
| 4   | Anagyrine | C_{20}H_{24}N_{2}O_{4} | Cr, Ct | [20, 85, 86] |
| 5   | Sparteine | C_{20}H_{24}N_{2}O_{4} | Cr, Ct | [25] |
| 6   | N-methylcytisine | C_{12}H_{18}N_{2}O | Cr, Ct | [20, 85–87] |
| 7   | Cytisine | C_{12}H_{18}N_{2}O | Cr, Ct | [25, 86] |
| 8   | 5,6-Dehydro-Î±-isolupanine | C_{11}H_{14}N_{2}O | Cr, Ct | [20, 85] |
| 9   | Lupanine | C_{20}H_{24}NO_{5} | Cr, Ct | [25, 86] |
| 10  | Baptifoline or Argentamin | C_{19}H_{21}NO_{5} | Ct | [85, 86] |
| 11  | O-acetylbaptifolin | C_{19}H_{21}NO_{5} | Ct | [20] |
| 12  | Î±-isolupanine | C_{19}H_{21}NO_{5} | Ct | [25, 86] |
| 13  | Thalictroidine | C_{14}H_{18}NO_{2} | Ct | [20, 25] |
| 14  | Caulophyllumine A | C_{13}H_{18}NO_{4} | Ct | [20] |
| 15  | Caulophyllumine B | C_{13}H_{18}NO_{4} | Ct | [20] |
| 16  | Piperidylacetophenone | C_{13}H_{18}NO_{4} | Ct | [20] |
| 17  | Caulophyne | C_{19}H_{21}NO_{5} | Cr | [88] |
| 18  | Caulophyline A | C_{12}H_{18}NO_{3} | Cr | [13] |
| 19  | Caulophyline B | C_{12}H_{18}NO_{3} | Cr | [13] |
| 20  | Caulophyline C | C_{12}H_{18}NO_{3} | Cr | [13] |
| 21  | Caulophyline D | C_{12}H_{18}NO_{3} | Cr | [13] |
| 22  | Caulophyline E | C_{12}H_{18}NO_{3} | Cr | [13] |

*Cr means C. robustum; Ct means C. thalictroides.*
Caulophyllum, namely, oleanolic acid (AG₁), hederagenin (AG₂), echinocystic acid (AG₃), and caulophylogenin (AG₄). However, Ma et al. reported 47–53 with abnormal sapogenins AG₁ to AG₁₁ from blue cohosh for the first time. As follows, 54 bearing sapogenin erythrodiol was discovered from genus Caulophyllum in 2012 [31]. These aglycones are closely related oxygenated pentacyclic triterpenoid structures that can be distinguished only by the positions and numbers of the double bonds in rings C and D and oxygenation patterns in positions C-16, C-23, and C-28. A possible biosynthetic pathway of Caulophyllum sapogenins can be hypothesized, as shown in Scheme 2. The first is, that 2,3-oxidosqualene is cyclized to the pentacyclic oleane-type triterpenoid backbone β-amyrin by plant oxidosqualene cyclases β-amyrin synthase [32, 33].

### Table 2: Chemical structures of triterpene saponins (23–54) from genus Caulophyllum.

| No. | Compound names | C-3 | C-28 | Formula | Sources* | References |
|-----|----------------|-----|------|---------|----------|------------|
| 23  | Ara → 3β-O-AG₁ | S₁  | —    | C₃₅H₅₆O₈ | Ct       | [21]       |
| 24  | Saponin PE, Glc → 2'Ara → 3β-O-AG₁ | S₂  | —    | C₄₁H₆₄O₁₂ | Ct       | [20, 21]   |
| 25  | Ciwujianside A, Glc → 2'Ara → 3β-O-AG₁-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₂, S₃ | C₉₉H₉₆O₂₆ | Ct | [20, 21] |
| 26  | Cauloside A, Ara → 3β-O-AG₂ | S₁  | —    | C₃₅H₅₆O₈ | Cr, Ct   | [20, 21, 30, 31, 37, 89] |
| 27  | Cauloside C, Glc → 2'Ara → 3β-O-AG₂ | S₂  | —    | C₄₁H₆₄O₁₃ | Cr, Ct   | [20, 21, 30, 37, 89] |
| 28  | Cauloside D, Ara → 3β-O-AG₂-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₁, S₃ | C₃₅H₅₆O₂₂ | Cr, Ct | [20–22, 30, 90] |
| 29  | Cauloside G, Glc → 2'Ara → 3β-O-AG₂-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₂, S₃ | C₉₉H₉₆O₂₇ | Cr, Ct | [20–22, 30, 91] |
| 30  | Cauloside b, Rha → 2'Ara → 3β-O-AG₂ | S₁  | —    | C₄₁H₆₄O₁₂ | Cr       | [92]       |
| 31  | Cauloside c, Ara → 2'Rha → 2'Ara → 3β-O-AG₂ | S₅  | —    | C₄₆H₅₄O₁₆ | Cr       | [92]       |
| 32  | AG₂-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | —    | S₁   | C₄₇H₇₆O₁₈ | Ct       | [21]       |
| 33  | Glc → 3'Ara → 3β-O-AG₂-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₆, S₃ | C₉₉H₉₆O₂₇ | Ct | [22, 93] |
| 34  | Ara → 3β-O-AG₂-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₁, S₅ | C₃₅H₅₆O₂₂ | Ct | [30]       |
| 35  | Ara → 3β-O-AG₂ | S₁  | —    | C₃₅H₅₆O₈ | Cr, Ct   | [21, 31, 37] |
| 36  | Glc → 2'Ara → 3β-O-AG₂ | S₂  | —    | C₄₁H₆₄O₁₃ | Ct       | [21, 30]   |
| 37  | Leiyemudanoside C, Glc → 3'Ara → 3β-O-AG₂-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₆  | S₅ | C₹₉H₉₆O₂₇ | Cr | [36]       |
| 38  | Ara → 3β-O-AG₂-28-O ↔ Glc⁶ ↔ Glc | S₆, S₇ | C₄₇H₇₆O₁₉ | Ct | [21]       |
| 39  | Glc → 2'Ara → 3β-O-AG₂-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₂, S₃ | C₉₉H₉₆O₂₇ | Ct | [30]       |
| 40  | Cauloside B, Ara → 3β-O-AG₄ | S₁  | —    | C₃₅H₅₆O₉ | Cr, Ct   | [20–22, 30, 31, 37, 94] |
| 41  | Leonticine D, Ara → 3β-O-AG₄-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₁, S₃ | C₃₅H₆₀O₂₃ | Ct | [21, 22, 30] |
| 42  | Cauloside H, Glc → 2'Ara → 3β-O-AG₄-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₂, S₃ | C₹₉H₉₆O₂₈ | Ct | [20]       |
| 43  | Leiyemudanoside A, Ara → 3β-O-AG₂-28-O ↔ Glc⁶ ↔ Glc | S₅, S₇ | C₄₇H₇₆O₁₉ | Cr | [36]       |
| 44  | Leiyemudanoside B, Glc → 3'Ara → 3β-O-AG₄-28-O ↔ Glc⁶ ↔ Glc⁴ ↔ Rha | S₆, S₅ | C₹₉H₉₆O₂₈ | Cr | [36]       |
| 45  | Glc → 2'Ara → 3β-O-AG₄ | S₂  | —    | C₄₁H₆₄O₁₄ | Ct       | [21]       |
| 46  | Ara → 3β-O-AG₄-28-O ↔ Glc | S₁, S₈ | C₄₁H₆₄O₁₄ | Ct | [21]       |
| 47  | Ara → 3β-O-AG₅ | S₁  | —    | C₃₅H₆₀O₈ | Ct       | [21]       |
| 48  | Ara → 3β-O-AG₆ | S₁  | —    | C₃₅H₆₀O₉ | Ct       | [21]       |
| 49  | Glc → 2'Ara → 3β-O-AG₇ | S₂  | —    | C₄₁H₆₄O₁₄ | Ct | [21]       |
| 50  | Ara → 3β-O-AG₇ | S₁  | —    | C₃₅H₆₀O₁₀ | Ct       | [21]       |
| 51  | Ara → 3β-O-AG₈ | S₁  | —    | C₃₅H₆₄O₉ | Ct       | [21]       |
| 52  | Glc → 2'Ara → 3β-O-AG₉ | S₂  | —    | C₄₁H₆₄O₁₃ | Ct | [21]       |
| 53  | Glc → 2'Ara → 3β-O-AG₁₀ | S₂  | —    | C₄₁H₆₄O₁₂ | Ct | [21]       |
| 54  | Ara → 3β-O-AG₁₂ | S₁  | —    | C₃₅H₆₀O₄ | Cr       | [31]       |

*Cr means C. robustum; Ct means C. thalictroides.
The β-amyrin experienced hydroxylation at C-28 to produce erythrodiol (AG\textsubscript{12}). Erythrodiol is further oxidized at the C-28 position by a single cytochrome P450 enzyme to yield oleanolic acid (AG\textsubscript{1}) [34]. The aglycones AG\textsubscript{1}–AG\textsubscript{9} may be derived from the common skeleton of oleanolic acid as precursors that firstly experience selective oxidation at C-23, or C-16, or both of C-23 and C-16 to afford hederagenin (AG\textsubscript{2}), echinocystic acid (AG\textsubscript{3}), and caulophyllogenin (AG\textsubscript{4}), respectively. Hederagenin may selectively involve a complex process such as dehydrogenization, oxidation, lactonization,
Scheme 2: Hypothesized biosynthetic pathway for oleanane aglycones from genus Caulophyllum. A series of step-by-step actions from 2,3-oxidosqualene to β-amyрин, erythrodiol, oleanolic acid, and other aglycones are assumed. Dehydration, and lactone ring hydrolysis to form diverse aglycones in genus Caulophyllum. Though the intermediate (3β,12α-dihydroxy-olean-28-oic γ-lactone) has been artificially synthesized from oleanolic acid (Supplementary Scheme 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2014/684508) [35], this type of biosynthetic pathway in plants also needs to be further confirmed.

The according carbohydrate chains are composed mainly of arabinose, rhamnose, and glucose moieties. After acid hydrolysis, gas chromatography analysis revealed the presence of glucose, arabinose, and rhamnose through comparing...
2.3. Other Compounds. Other minor compounds are present in this genus, such as fatty acids and sterols [37, 38]. These compounds were identified as palmitic acid (55), α-spinasterol (56), α-spinasterol-β-D-glucopyranoside (57), stigmasterol (58), lupeol (59), and cholesterol (60) (Figure 3). Diverse aglycones, monosaccharide residues, and diverse linkage mode of sugar moieties are possible to form diverse structures of triterpene saponins from genus Caulophyllum.

3. Synthetic Taspine Derivatives

Zhang et al. designed and synthesized four novel ring-opened target compounds (1’–4’) by structure-based drug design. This design includes two pathways: cleavage of the C–C bond of diphenyl and ester bond of ring B and ring D (Scheme 3(A)). Targeted compounds 1’ and 2’ were synthesized by the route outlined in Scheme 3(B). Isovanillin (5’) was used as the starting material, which was firstly oxidized to afford isovanillic acid (6’). The methyl ester 7’ was prepared to avoid side-reactions of the carboxylate group. Then refluxing of 7’ with prenyl bromide in the presence of K$_2$CO$_3$ in anhydrous acetone afforded 8’. A solution of 8’ in N,N-dimethylaniline was heated to reflux to give 9’. Prenyl group was moved into the paraposition of hydroxyl in Claisen rearrangement process. The next step was the coupling of 9’ to the carboxyl of 10’ by an ester bond with DCC and DMAP. The oxidation of 11’ produced aldehyde 12’, which reacted with dimethylamine followed by reduction to give 13’. At last, benzyl deprotection of 13’ with palladium-carbon in MeOH gave 1’. The 3’ and 4’ were synthesized in the same way from isovanillin [17].

Synthetic endeavors into cleavage of the C–C bond and ester bond of rings B, D, and E have been studied (Scheme 4(A)). Initially, six target biphenyl derivatives (19’–24’) were successfully synthesized by general routes described in Scheme 4(B) employing a classical symmetrical Ullmann reaction [18]. Isovanillin (5’) was also used for the starting material, which was required for seven steps to afford 19’ by bromination, benzyla- tion, oxidation, substitution reaction, Ullmann reaction, and catalytic hydrogenation. 19’ is an important intermediate to synthesize the following targeted compounds. During the synthesis of unsymmetrical biphenyl (22’), a novel symmetrical biphenyl derivative (31’) was surprisingly isolated as a byproduct [41], which exhibited potent anticancer activity to attract increasing attention. To further investigate this finding, researchers aimed to enhance the structural complexity and diversity of 22’ by generating novel biphenyls (Figure 4) [41]. As a result, eighteen symmetrical biphenyls derivatives (31’–48’) were firstly prepared [42]. Following these, He et al. used 20’ as the identifying group and synthesized another two novel taspine diphenyl derivatives 49’ and 50’, which were made by introducing coumarin groups into the structure of 20’ [43]. Meanwhile, derivatives 51’ and 52’ were obtained via similar procedures (Scheme 4(B)) [44].
4. Bioactivity

4.1. Antibacterial Activity. Earlier biological studies showed that caulosides A–D and G (26, 40, 27–29) have antimicrobial activity [45]. Recently, triterpene compounds isolated from C. robustum showed microorganism inhibitory activities to the test fungi and bacteria. Moreover, compound 35 and cauloside B (40) had notable inhibiting microorganism activities to bacteria with minimal inhibitory concentration (MIC) of 3.9 μg/mL [11]. Ethanol extract and its five subfractions of C. robustum showed high antibacterial activity against Staphylococcus aureus, Staphylococcus aureus (clinic bacterial), and...
4.2. Anti-Inflammatory and Analgesic Effects. The anti-inflammatory and analgesic effects of ethanol extract, chloroform extract, and n-butyl alcohol extract from C. robustum were observed by several animal experiments. Among the different organic extracts, the action of alcohol extract was better than other organic extracts [12]. Cauloside A (26) and cauloside C (27) had anti-inflammatory and analgesic activities at dose dependency and the analgesic effect was the most significant when compounds were injected for 30 min [11]. From the points of structure-activity relationship of the saponins, cauloside C (27) with disaccharide has more potent analgesic effect than cauloside A (26) with monosaccharide. Oppositely, cauloside A (26) has more potent anti-inflammatory activity than cauloside C (27). The anti-inflammatory activity of taspine hydrochloride has been demonstrated by using the carrageenan-induced pedal edema method, the cotton pellet-induced granuloma method, and the adjuvant polyarthritis model [47].

Lee et al. (2012) assessed the *in vitro* and *in vivo* effects of blue cohosh on lipopolysaccharide (LPS)-induced cytokines in BV2 cells and mice. Several lines of evidence indicate that blue cohosh treatment suppressed the elevation of LPS-induced iNOS (inducible nitric oxide synthase) expression in a concentration-dependent manner in microglia cells. Blue cohosh saponins (caulosides A–D: 26, 40, 27, 28) significantly suppressed the expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6. In addition, blue cohosh extract suppressed the expression of COX (cyclooxygenase)-2, iNOS, and proinflammatory cytokines in adrenal glands of mice. So, it is concluded that saponin constituents of blue cohosh exert anti-inflammatory effects through the inhibition of expression of iNOS and proinflammatory cytokines [48].

4.3. Antioxidant Effects. Caulophylline A–D (18–21) afforded the lower scavenging effects against DPPH (1,1-diphenyl-2-picrylhydrazyl) radical at test concentration (6.0 to 107.4 μg/mL). Caulophylline E (22) showed good scavenging effects against DPPH radical with IC₅₀ (half-inhibition concentration) of 12.1 μg/mL [13]. Antioxidant activities of the

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**Scheme 4:** (A) Design of ring-opened target compounds 19’–24’; (B) preparation of target compounds (19’–24’). Reagents and Conditions: (a) Fe, NaOAc, AcOH, Br₂, 81%; (b) BnCl, K₂CO₃, 95%; (c) NaH₂PO₄, NaClO₂, 30% H₂O₂, 93%; (d) SOCl₂, DMF(cat), CH₂Cl₂, 96%; (e) CH₂Cl₂, 30% CH₂NH₂, 85%; (f) Cu, DMF, 72%; (g) H₂, Pd/C, 97%; (h) K₂CO₃, DMF, (26, 64%; 25, 76%); (i) K₂CO₃, EtOH, (27, 72%; 28, 59%; 29, 54%) [18, 42–44].

**Bacillus subtilis**, and the diameters of the biggest inhibition zone were 20.03 mm, 23.52 mm, and 20.77 mm. The MICs of these were 0.31–0.63 mg/mL [46].
polysaccharide fraction, ethanol extract, and different polar fractions of *C. robustum* were evaluated by DPPH, hydroxyl, and superoxide radical and nitrogen dioxide (NO\(_2\)) scavenging assay [39, 49]. The results showed that ethanol extract and different polar fractions displayed high antioxidant activities. The scavenging activities of polysaccharides from *C. robustum* for DPPH, hydroxyl, and superoxide radical and NO\(_2\) were attributed to 80%, 96%, 78%, and 85.1%, respectively, for the concentrations of 5.0 mg/mL. Another experiment research reported that chloroform partition fraction showed IC\(_{50}\) value of DPPH-free radical-scavenging activity which was 79.4 \(\mu\)g/mL [14].

4.4. Antiacetylcholinesterase Activity. As early as 2006, taspine (2) has been confirmed to be an antiacetylcholinesterase (AChE) inhibitory agent by a bioactivity-guided approach in a *Magnolia x soulangiana* extract using a microplate enzyme assay with Ellman’s reagent [50]. 2 showed a significantly higher effect on AChE than the positive control galantamine and selectively inhibited the enzyme in a long-lasting and concentration-dependent fashion with an IC\(_{50}\) value of 0.12 \(\mu\)g/mL. It could be suggested that taspine might be a potential candidate for the development of anti-AD (Alzheimer’s disease) treatment.

More recently, *C. robustum* has been confirmed to possess significant AChE activity with inhibition rates (88.72 \(\pm\) 1.47)% at the concentration of 1 g L\(^{-1}\) through thin layer chromatography bioautographic method. Furthermore, chloroform fractions have shown higher AChE inhibitory capacity, so it will be further performed bioguided isolation and purification to obtain active compounds [14]. In addition to taspine in *C. robustum*, whether to have other compounds responsible for the activity of AChE is worthy of studying further.

4.5. Effect on Atherosclerosis and Myocardial Ischemia. It was found that the n-butanol fraction of *C. robustum* was an effective part, and caulophine (17) separated from the part was an active one in vasodilatation [51, 52]. The n-butanol fraction may have protective action on H\(_2\)O\(_2\) injured-human umbilical vein endothelial cell line *in vitro*, and its mechanism of action may be related to the increase of the level of nitric oxide (NO), NOS (nitric oxide synthase), and the expression of NF-\(\kappa\)B (nuclear factor kappa B) [53]. The interaction between

![Figure 4: Structures of 31°–51°.](image-url)
the effective component \( m \) and the membrane or membrane receptor was reflected in the vascular CMC model, which suggested that \( m \) may exert bioactivity in the heart [52]. The deeper study demonstrates that \( m \) is able to protect cardiomyocytes from oxidative and ischemic injury through an antioxidative mechanism [54] and from caffeine-induced injury via calcium antagonism [51].

4.6. Antitumor Activity and Mechanism of Action. The cytotoxicity (IC\(_{50}\)) of taspine (2) was found to be 0.39 \( \mu \)g/mL against KB cells and 0.17 \( \mu \)g/mL against V-79 cells [55]. 2 showed antitumor activity on the mouse S180 sarcoma in a good dose-dependent manner [56]. The inhibition rates on tumor of taspine at low, middle, and high concentrations were 39.08\%, 43.99\%, and 48.60\%, respectively. The microvessel density and protein expressing of the vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), Bcl-2, and Bax in the tumor were decreased compared with the negative control. The ratio of Bax to Bcl-2 was increased. 2 has antitumor effect on the S180 sarcoma, and the mechanism may be through the way of decreasing the expressing of the VEGF, bFGF, Bcl-2, and Bax and inducing the vascular endothelial cell apoptosis.

Zhan et al. was to investigate the effect of taspine on the growth of oestrogen-receptor-positive breast cancer xenografts in vivo and the possible mechanism for this action [19]. Cell cycle and apoptosis analysis documented that taspine was able to change cell cycle and induce cell apoptosis. There was a significant decrease in the expression of estrogen receptor (ER) and progesterone receptor (PR) both in tumor tissue and cells after treatment with taspine. At the same time, it also showed a reduction in the expression of mRNA for ER and PR in the group treated with taspine. These data suggested that taspine might serve as a promising candidate of ER antagonist in the treatment of oestrogen-independent breast cancer.

Many evidences have shown that taspine could suppress tumor-induced angiogenesis. Taspine was able to inhibit chicken chorioallantoic membrane angiogenesis through interfering with the proliferation and migration of endothelial cells in a dose-dependent manner [57]. The exact mechanism [15] has been further demonstrated, suggesting that VEGF and bFGF secretion were downregulated by taspine in human non-small cell lung cancer cell (A549) cell and human umbilical vein endothelial cells (HUVECs), confirmed by the decreased mRNA level of VEGF and Flk-1/KDR after taspine treatment in HUVECs. The molecular mechanisms of taspine on tumor angiogenic inhibition have been further studied in vitro [58], which indicated that taspine significantly inhibited cell proliferation of HUVECs induced by VEGF165 via decreasing Akt and Erk1/2 activities except decreasing VEGF level. Authors assume that taspine can inhibit the proliferation of vascular endothelial cells in tumor by regulating P13 kinase and MAP kinase signal pathways.

Additionally, taspine could induce apoptosis of HUVECs in a dose-dependent manner [59]. Cell cycle was significantly stopped at the S phase. The morphology of HUVEC treated with taspine showed nuclear karyopycnosis, chromatin agglutination, and typical apoptotic body detected by electronic microscope. Taspine has an inhibitory effect on growth of HUVECs and can induce its apoptosis by decreasing Bcl-2 expression and increasing bax expression. Zhang et al. continuously investigated the effects of taspine on the proliferation and apoptosis in the A431 cell [60]. The cell cycle was significantly stopped at S phase, and nuclear karyopycnosis, chromatin agglutination, and typical apoptotic bodies were found after taspine treatment in A431 cells. There was a decrease in the expression of Bcl-2, whereas the expression of caspase-3, cleaved caspase-3, CDK2, CDK4, and Bax increased. These data demonstrated that taspine can induce apoptosis by activating caspase-3 expression and upregulating the ratio of Bax/Bcl-2 in A431 cells.

The preliminary biological test demonstrated that derivative 3 showed much better inhibitory activities against CACO-2 (IC\(_{50}\) = 0.023 \( \mu \)g/mL) and ECV304 (IC\(_{50}\) = 0.0012 \( \mu \)g/mL) than taspine [17]. A deep research demonstrated that most derivatives (1’-4’ and 19’-24’) possessed a moderate degree of cytotoxicity against human cancer cell lines [18]. One of them (3’) exhibited much better antiangiogenic activity against CACO-2 (IC\(_{50}\) = 23.4 \( \mu \)g/mL) and ECV304 (IC\(_{50}\) = 1.19 \( \mu \)g/mL) than taspine did. Some of the compounds showed good antiangiogenic activity against colon (HT29), breast (MCF-7), lung (A549), rectum (CACO-2), skin (A375), hepatoma (7272), and pancreatic (PANC-1) cancers cell lines. A continual research demonstrated that derivative 3‘ can inhibit the proliferation of, and induce apoptosis in, Caco-2 cells by activating caspase-3, caspase-8, and caspase-9, downregulating the expressions of VEGF, and upregulating the ratio of bax/bcl-2 [61].

Derivatives (31’ and 32’) demonstrated the most potent cytotoxic activity with IC\(_{50}\) values between 14.2 \( \mu \)g/mL and 22.3 \( \mu \)g/mL among symmetrical taspine derivatives (31’-48’) [42]. Biphenyls without halogen substitution (34’, 38’, 39’, and 44’) were much less potent than those containing halogen. Halogen substitution played a critical role in the activity of biphenyls. Derivative 31’ inhibits tumor growth in xenographed A549 cells in nude mice by inhibiting the growth of neovessels. In other words, derivative 31’ is an inhibitor of angiogenesis which functions by downregulating VEGF [62]. Furthermore, derivative 31’ had potential to suppress the adhesion, migration, and invasion of ZR-75-30 cancer cells, and it could serve as a potential novel therapeutic candidate for the treatment of metastatic breast cancer [63]. Derivative 48’ could inhibit proliferation of lovo cell and tumor growth in a human colon tumor xenographed model of athymic mice, which might be a novel angiogenesis inhibitor that reduces angiogenic responses in vivo and in vitro by blocking VEGFR signaling pathways [64].

Two novel derivatives (49’ and 50’) by introducing different coumarin fluorescent groups into the basic structure have not only fluorescence but also the ability to inhibit effects on different breast cancer cell lines, which indicates their possible further use as dual functional fluorescence probes in tracer analysis. Derivative 51’ inhibits tumor growth and cell proliferation by inhibiting cell migration, downregulating mRNA expression of VEGF and EGF, and decreasing angiogenic factor production, which deserves further consideration as a chemotherapeutic agent [44]. All evidences
have demonstrated that the lactone ring B is important for activity, while the lactone ring D can be opened, thus retaining and even improving the antiproliferative properties of taspine. Halogen substitution could potentially improve the anticancer activity of the biphenyl derivatives [17, 18, 42].

4.7. Inhibitory Cytochrome P450 Effects. The methanolic extracts of the roots of blue cohosh, the alkaloid fraction, and isolated constituents were evaluated for their inhibition of major drug metabolizing cytochrome P450 (CYP450) enzymes [65]. The methanolic extracts did not show any effect but the alkaloid fraction showed a strong inhibition of CYP2C19, 3A4, 2D6, and 1A2 (>80% inhibition at 100 μg/mL) with IC₅₀ values in the range of 2–20 μg/mL. Among the isolated alkaloids, caulophylcline B (15), O-acetyl baptifolin (11), anagyrine (4), and lupanine (9) inhibited these enzymes to various extents (IC₅₀: 0.5–15.1 μg/mL). N-methylcytisine (6) showed weak activity against the CYP3A4 in vitro with 32% inhibition at 20.4 μg/mL. An equimolar mixture of alkaloids exhibited a more pronounced inhibitory effect on all four enzymes as compared to the isolated alkaloids. Among the saponins, caulosides C (27) and D (28) showed 43% and 35% inhibition of CYP3A4 at the concentration of 76.6 and 107.4 μg/mL, respectively. Other enzymes were not affected. This in vitro study indicates that dietary supplements containing blue cohosh may pose a risk of drug-drug interactions if taken with other drugs or herbs, metabolism of which involves CYP450 enzymes.

4.8. Topoisomerase Inhibitor. Taspine (2) was found to induce conformational activation of the proapoptotic proteins Bak and Bax, mitochondrial cytochrome c release, and mitochondrial membrane permeabilization in HCT116 cells [66]. Analysis of the gene expression signature of taspine treated cells suggested that taspine is a topoisomerase inhibitor. Taspine has a reduced cytotoxic effect on a cell line with a mutated topoisomerase II enzyme. Interestingly, in contrast to the topoisomerase II inhibitors doxorubicin, etoposide, and mitoxantrone, taspine was cytotoxic to cell lines overexpressing the Pgp or MRP drug efflux transporters. Taspine induces widespread apoptosis in colon carcinoma multicellular spheroids and that apoptosis is induced in two xenograft mouse models in vivo. Taspine is a dual topoisomerase inhibitor that is effective in cells overexpressing drug efflux transporters and induces wide-spread apoptosis in multicellular spheroids.

4.9. Effect on Wound Healing. A patent reported that the method is useful for preparing wound care composition, which comprises C. robustum, which is useful for relieving postoperative pain and promoting wound healing and blood circulation in wound area [67]. Further research showed that taspine was able to promote early phases of wound healing in a dose-dependent manner with no substantial modification thereafter. Its mechanism of action is probably related to its chemotactic properties on fibroblasts and is not mediated by changes in extracellular matrix [68]. Authors summarized that taspine opens a pathway of research for new tools to stimulate wound repair in the absence of macrophages, thereby helping to better understand the process of wound healing. Taspine also exhibited a dose-related cicatrizing effect and a median effective dose (ED₅₀) of 0.375 mg/kg, which was nontoxic to human foreskin fibroblasts at concentrations below 150 ng/mL and that had no effect on cell proliferation [69].

4.10. Toxicity. N-methylcytisine (6) exhibited teratogenic activity in the rat embryo culture (REC), an in vitro method to detect potential teratogens. Anagyrine (4) and α-isolupanine (12) were not teratogenic in the REC at tested concentrations. Taspine (2) showed high embryotoxicity, but no teratogenic activity, in the REC [25]. Wu et al. have observed that blue cohosh interrupted medaka embryogenesis and produced an abnormal phenotype, which identifies blue cohosh as a potent teratogen. Moreover, the induction of gata2 mRNA followed by edn1 mRNA by BC indicates that the teratogenic response of blue cohosh is probably mediated by the Gata2-End1 signaling pathway [9]. Caulosides B (40) and C (21) were reported to have cytotoxicity to developing sea urchin embryos by changing cell permeability. It is well-known that cytotoxic glycoside causes a disturbance of cell membrane permeability that can cause leakage of important cellular components [70, 71].

A new born infant whose mother ingested an herbal medication, blue cohosh, to promote uterine contractions presented with acute myocardial infarction associated with profound congestive heart failure and shock [7]. One year later, other similar cases were reported [72]. Meanwhile, According to a survey of midwives in the United States, approximately 64% of midwives reported using blue cohosh as a labour-inducing aid. Severe multiorgan hypoxic injury may occur. Recently, a review focused on the toxicity of blue cohosh has been reported [2].

5. Pharmacokinetics

Magnoflorine (1), taspine (2), and caulophine (17) were the main components of genus Caulophyllum. Several studies have been carried out to understand the distribution, absorption, metabolism, and excretion of magnoflorine (1), taspine (2), and caulophine (17) using modern analytical methods.

5.1. Pharmacokinetics of Magnoflorine. As far as magnoflorine is concerned, a new sample-preparation method based on hollow-fiber liquid-phase microextraction (HFLPME) was developed and successfully used for pharmacokinetic studies of magnoflorine in rat plasma after intravenous administration. The magnoflorine disappears from rat plasma in accordance with a two-compartment open model. The plasma concentration of magnoflorine reached a peak immediately after completion of administration, then began to decline. Without doubt, the chromatographic and HFLPME sample-preparation procedures of magnoflorine will facilitate the development and validation of other methods of analysis of magnoflorine in other biological matrices [73].
5.2. Pharmacokinetics of Taspine. Lu et al. (2008) prepared taspine solid lipid nanoparticles (2-SLN) and taspine solid lipid nanoparticles with galactoside (2-G2SLN) separately using the film evaporation extrusion method. The pharmacokinetics and liver target efficiency after IV administrations of 2-SLN and 2-G2SLN to ICR mice were finally compared [59]. The pharmacokinetics and tissue distribution after intravenous administrations of taspine solution and taspine liposome to ICR mice were compared. Incorporation into liposomes prolonged taspine retention within the systemic circulation and increased its distribution to the spleen and liver but reduced its distribution to the heart and brain [74].

5.3. Pharmacokinetics of Caulophine. Pharmacokinetic studies have shown that caulophine (17) is easily absorbed after oral administration, but it is eliminated from the body slowly. In fact, 1.25 h after treating rats treated with caulophine, the highest concentration of caulophine was found in the liver. Therefore, hepatic metabolism is probably the main route for the in vivo processing of caulophine [75]. Two metabolites including glucuronide conjugate and N-oxide of caulophine were found in rat urine and feces by HPLC-MS. Moreover, the same caulophine glucuronide conjugate was observed in rat liver microsomes system. However, caulophine glucuronide conjugate was not observed in dog liver microsomes [28].

6. Cell Membrane Chromatography for Activity Screening

Cell membrane chromatography (CMC) is a novel bioaffinity chromatographic technique. The CMC combined with high performance liquid chromatography (HPLC) or HPLC/MS will be of great utility in drug discovery using natural medicinal herbs as a source of novel compounds. In reported studies, the model of CMC in which cell membrane is enriched with certain receptors is used, as the stationary phase was applied to screen the target components from medicinal herbs as a source of novel compounds. In reported studies, the cell membrane chromotography (CMC) model of CMC in which cell membrane is enriched with certain receptors is used, as the stationary phase was applied to recognize, separate, and identify target components from medicinal herbs [76–78] and to investigate the interactions between drug and receptor [79, 80]. This system has been successfully applied to the screening and identification of active components from C. robustum.

A combined A431/CMC-HPLC method was developed and was successfully applied to recognize, separate, and identify target components “taspine” and “caulophine” from C. robustum [81]. A combined A431/CMC with online HPLC/MS was also established for identifying active components from C. robustum acting on human epidermal growth factor receptor (EGFR) [77]. Retention fractions on A431/CMC model were captured onto an enrichment column and the components were directly analyzed by combining a 10-port column switcher with an LC/MS system for separation and preliminary identification. Using sorafenib tosylate as a positive control, taspine (2) and caulophine (17) were identified as the active molecules which could act on the EGFR. Other research results showed that taspine (2) was the active molecule acting on the tumor vasodilatation [52], and magnoflorine (1) and caulophine (17) were the active molecules acting on the human α1A-adrenoceptor (α1A AR) [82].

This system has been also successfully applied to investigate the interactions between active compounds from C. robustum and receptor. A new high-expression vascular endothelial growth factor receptor-2 (VEGFR-2) CMC method combined with mathematical treatments was proposed for evaluating taspine-receptor interactions [83]. A competitive binding study was performed and the results indicate that there are multiple types of binding sites on VEGFR-2 for taspine (2). Following this, Du and coworkers developed another new high-expression EGFR CMC method to recognize the ligands acting on EGFR specifically and investigate the affinity of gefitinib/a novel taspine derivative HMQ1611 to EGFR [84]. It has been proven that the CMC method combined zonal elution provides a powerful technique for the characterization of HMQ1611 binding to the EGFR.

7. Conclusions and Future Prospects

The present review discuss the chemistry and pharmacological aspects of the genus Caulophyllum and especially provides a detailed analysis of the literature published since the year of 2000. The state of the science on Caulophyllum chemistry and pharmacological activity leaves considerable opportunity for future discoveries.

Two new classes of alkaloids, piperidine-acetophenone conjugates (13–16) and fluorenone (17–22) alkaloids, have been reported from genus Caulophyllum, suggesting that piperidine-acetophenone conjugates and fluorenone type alkaloids are another two major kinds of metabolites that existed in this genus Caulophyllum. In addition to common aglycones (oleanolic acid, hederagenin, echinocystic acid, and caulophyllogenin), eight other kinds of aglycones have been found from Caulophyllum species. Diverse aglycones, monosaccharide residues, and linked modes of sugars are possible to form diverse structures of triterpene saponins from genus Caulophyllum. Many new compounds have been identified in recent years, and we are convinced that more trace constituents with novel structures will be discovered with the development of new technology for isolation and identification.

Currently, although many purified compounds have been tested for activity which are 1, 2, 4, 5, 6, 9, 11, 15, 17, 26–29, 35, and 40, only 2 (taspine) is performed in-depth study on its anti-tumor and anti-angiogenic mechanisms and could serve as a lead compound in anticancer agent development. Meanwhile, a class of biphenyl derivatives of taspine was designed and synthesized for screening potential novel anticancer agents. Besides 2, caulophine (17) was identified as another active molecule which could act on the EGFR and α1A AR by combining α1A AR/CMC and A431/CMC with online HPLC/MS. 17 also merits further research to see its action of mechanisms. On the other hand, a number of compounds with novel structure skeleton, such as 13–16, 18–22, 50, 51, 52, 53, and 54 have previously been isolated, but no further tests have been performed. It is possible that these compounds are
usually overlooked due to their low abundance in *Caulophyllum*. Pharmacokinetic study is also limited for compounds isolated, mainly involving three active alkaloids 1, 2, and 17. So it is very urgent to develop pharmacokinetic study *in vivo* for other bioactive compounds in genus *Caulophyllum*.

Pharmacological studies carried out on crude extracts and pure metabolites provided pragmatic documents for its traditional uses and have revealed that this genus is a valuable source for medicinally important molecules. Many important biological activities of this genus have been demonstrated such as anti-inflammatory and analgesic effects, antioxidant effects, anticholinesterase activity, and antitumor et al. Though many promising results were confirmed by animal models, it should be further investigated by clinical trials. Regarding the constituents contributed to medicinal values, the findings indicated that alkaloids and triterpene saponins were regarded as the major constituents in this genus, while polysaccharides that occurred in the genus are worthy of further research by chemical and pharmacological activities [37]. However, most of the plant extracts used in the above bioassay were not well characterized, and this defect led to the difficulty to reproduce the reported results. To add the availability of primary experimental data, suitable analytical and standardization protocols of plant materials should be developed, since these are the ground work for convincing and reproducible pharmacological studies.

The toxicity of *Caulophyllum* species is not negligible, mainly involving the teratogenic effects and inducing heart failure and shock by ingesting blue cohosh. From the view of current research results, alkaloid fractions may be responsible for major toxicity. However, exact individuals are required for further research by chemical and pharmacological experiments. The future work should be focused on the relationship between clinical effects and side-effects of *Caulophyllum* extracts to screen a safe and effective dosage. Moreover, a strict quality control procedure should be adopted to guarantee its quality. On the other hand, the alkaloids and triterpene saponins are two major kinds of constituents in blue cohosh, which are easily divided by chromatography methods [22]. The individual pharmacological tests for fractions of alkaloids and triterpene saponins should be considered according to the traditional and modern uses of *Caulophyllum* plants. Whether alkaloids and triterpene fractions can be used separately in the future according to each medical function, it may be a good choice for *Caulophyllum* plants for reducing the drug interaction and enhancing their efficiency.

**Abbreviations**

- AChE: Antiacetylcholinesterase
- AG: Aglycones
- AR: Adrenoceptor
- Ara: α-L-Arabino-pyranoside
- Bfgf: Basic fibroblast growth factor
- CMC: Cell membrane chromatography
- COX: Cyclooxygenase
- CYP450: Cytochrome P450
- DPPH: 1,1-Diphenyl-2-picrylhydrazyl
- EGFR: Epidermal growth factor receptor
- ER: Estrogen receptor
- GC/MS: Gas chromatography/mass spectrum
- Glc: β-D-Glucopyranose
- HFLPME: Hollow-fiber liquid-phase microextraction
- HPLC: High performance liquid chromatography
- HPLC/MS: High performance liquid chromatography/mass spectrum
- HUVECs: Human umbilical vein endothelial cells
- IC₅₀: Half-inhibition concentration
- IL: Interleukin
- iNOS: Inducible nitric oxide synthase
- LPS: Lipopolysaccharide
- MIC: Minimal inhibitory concentration
- NF-κB: Nuclear factor kappa B
- NO: Nitric oxide
- NO₂: Nitrogen dioxide
- PR: Progesterone receptor
- REC: Rat embryo culture
- Rha: α-L-Rhamnopyranose
- TNF-α: Tumor necrosis factor-α
- TV: Tumor vasodilatation
- VEGF: Vascular endothelial growth factor
- VEGFR-2: Vascular endothelial growth factor receptor-2.

**Conflict of Interests**

The authors declare that they have no conflict of interests regarding the publication of this paper.

**Authors’ Contribution**

Yong-Gang Xia and Guo-Yu Li equally contributed to this work.

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