Review

Chemical constituents and pharmacological effects of *Croton crassifolius*

Luyan Yang[a], Yibo Wang[a], Xinyao Wang[b], Xiaoxia Xu[a], Yunpeng Yang[a], Meng Zhang[a]*, Yu Chen[a]*

[a] *School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, China;*
[b] *School of Business, Liaoning University, Shenyang 110136, China*

Abstract

Croton crassifolius **(CC) is a kind of traditional Chinese medicine rich in terpenoids, with among which diterpene species are the most. CC had biological activities in the treatment of cancer, inflammation, ulcerative colitis, osteoporosis and Alzheimer's disease. This study reviews the chemical compositions and pharmacological activities of CC to provide reference for further research.**

Keywords: *Croton crassifolius*; chemical compositions; pharmacological activities

1 Introduction

Croton crassifolius (*C. crassifolius*) is a plant of *Croton* genus (family Euphorbiaceae). It is widely distributed in Guangxi, Guangdong, and other parts of China. In traditional Chinese medicine, doctors use the root of *C. crassifolius* as the medicine to treat back pain, stomach pain, abdominal distension, rheumatism, jaundice and joint pain. Modern clinical studies have demonstrated the effectiveness of using *C. crassifolius* or compounds containing *C. crassifolius* to treat traumatic arthritis [1], helicobacter pylori infection [2] and other diseases. However, there are relatively few studies on *C. crassifolius*. In order to solve the problem, we conducted a literature search on the chemical substances and pharmacological activities of *C. crassifolius*, aiming to provide reference for the development of new drugs and the promotion of the traditional development of ethnic traditional medicine.

2 Chemical constituents

The chemical constituents of *C. crassifolius* are mainly terpenoids and flavonoids, as well as some amino acids and steroids. *C. crassifolius* is rich in terpenoids, including sesquiterpenes, diterpenes and triterpenes. Among them, diterpenes are the most. New compounds were frequently isolated and identified from *C. crassifolius* and these newly

* Author to whom correspondence should be addressed. Address: School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, China; Tel.: +86-18341400530 (Yu Chen), +86-18341400685 (Meng Zhang); E-mail: gzweishengwu@126.com (Yu Chen), zhangmeng123321@126.com (Meng Zhang).

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identified compounds were mainly terpenoids.

2.1 Terpene constituents

2.1.1 Sesquiterpene components

There are a few sesquiterpene species isolated from *C. crassifolius*, and new compounds have been identified in recent years, some of which have good biological activities. For example, cyperenoic acid has anti-angiogenic activity. The structures of these chemical components these chemical components are shown in Table 1 and Fig. 1.

Table 1 Sesquiterpene components of *C. crassifolius*

No.	Chemical component	References
1	cyperenoic acid	[3]
2	Eyperenol	[3]
3	ent-spathulenol	[3]
4	4,5-secoguaial(10),11-diene-4,5-dioxo	[3]
5	1*β*,11-dihydroxy-5-eudesmene	[3]
6	spathulenol	[3]
7	araradendrme diol	[3]
8	(4*S**,7*R**,8*R**,10*S**)-8-hydroxy-*α*-guaiene	[3]
9	(+)-guaial(10),11-dien-9-one	[3]
10	cracroson H	[3]
11	crossrassin A	[4]
12	crossrassin B	[4]
13	cyperenoic acid-9-*O*-*β*-D-glucopyranoside	[5]
14	4*β*,10*α*-aromadendranediol	[5]
15	aromadendrane-4*α*,8*α*,10*α*-triol	[5]
16	4*β*-dihydroxyaromdendrene	[5]

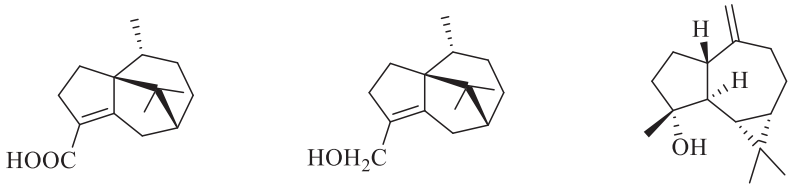

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Fig. 1 Structures of sesquiterpene components of *C. crassifolius*

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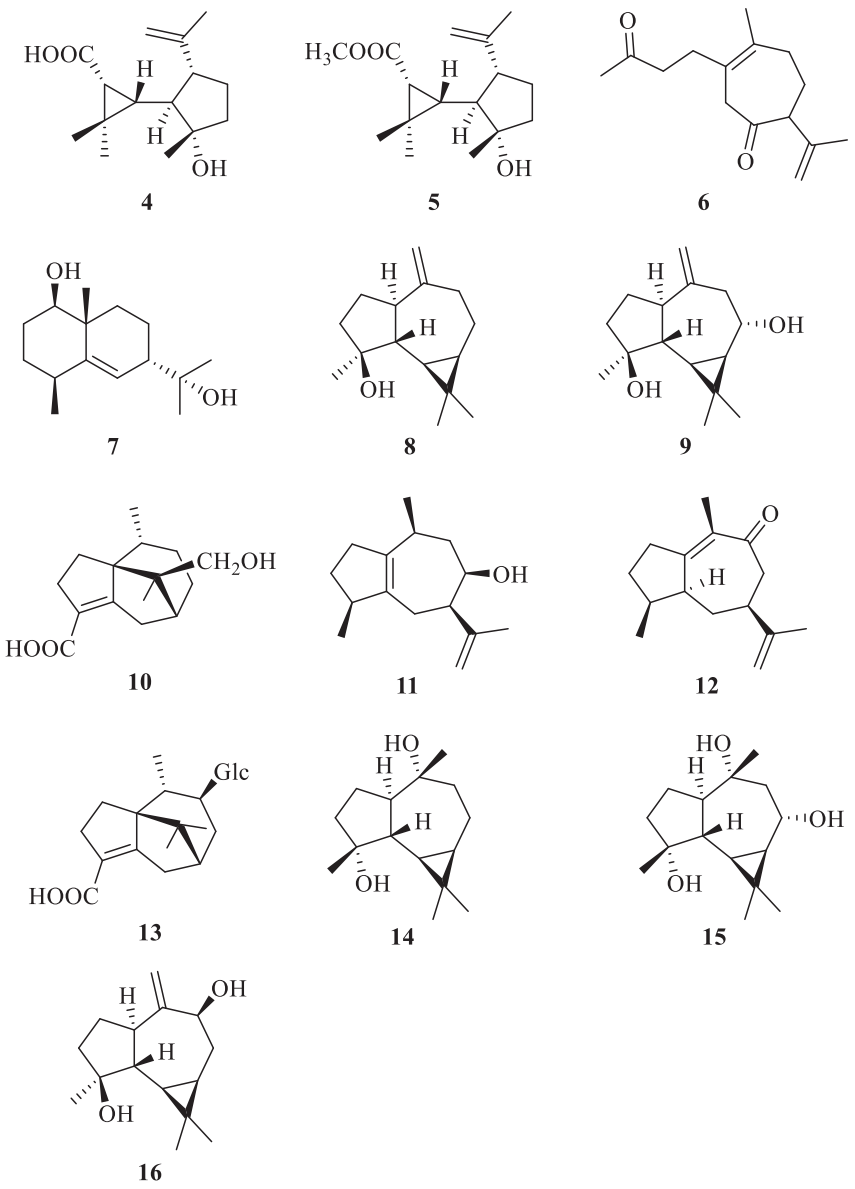

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2.1.2 Diterpenoid components

C. crassifolius is rich in diterpenes. These diterpenes possess the main pharmacological activities of *C. crassifolius*, and they contain several sites where chemical reactions occur to produce new compounds, which have great potential for the development of new drugs. Their structures are shown in Table 2 and Fig. 2.

Table 2 Diterpene compounds of *C. crassifolius*

No.	Chemical component	References
17-19	cracrasons A-C	[3]
20	mollotucin D dillactone este	[3]
21	penduliflaworosin	[3]
22	teucvin	[3]
23	1,4-methano-3-benzoxepin-2(1*H*)-one	[3]
24	teuscorolide	[3]
25	mallotucin D	[3]
26-27	chettaphanins I-II	[3]
28	9-[2-(2(5H)-furanone-4-yl)ethyl]-4,8,9-trimethyl-	[3]
29	1,2,3,4,5,6,7,8,9-octahydronaphthalene-4-carboxylic acid teucvidin	[3]
30	neoclerodan-5,10-en19,6*β*,20,12-diolide	[3]
31	methy[9-2(5*H*)-furanone-4-yl]-4,8,9-trimethyl-1,2,3,4,5,6.7,8-octahydronaphalene-4-carboxylic ester	[3]
32	spiro[furan-3-(2*H*),1'(2'*H*)-naphthalene]-5'-carboxylic acid	[3]
33-40	crassins A-H	[4]
41-63	crassifolins A-W	[5]
64	isoteufin	[6]
65	crassifoliusin A	[7]
66-67	mallotucins B-C	[8]
68	(12s)-15,16-epoxy-6*β*-methyoxy-19-norneoclerodane	[8]
69	5(10),13*E*-ent-halimandien-15,16-olide-19*α*-oic-acid	[8]
70	5(10),13*E*-ent-halimandien-15,16-olide-19*α*-oic acid methyl ester	[8]
71	crassifolius P	[9]
72	crassifolius Q	[9]
73	3*S*-methoxyl-teucvin	[10]
74	3*R*-methoxyl-teucvin	[10]
75-78	cracrosons D-G	[10]
79	norcrassin A	[11]
80	bicrotonol A	[11]
81	isoteucvin	[12]

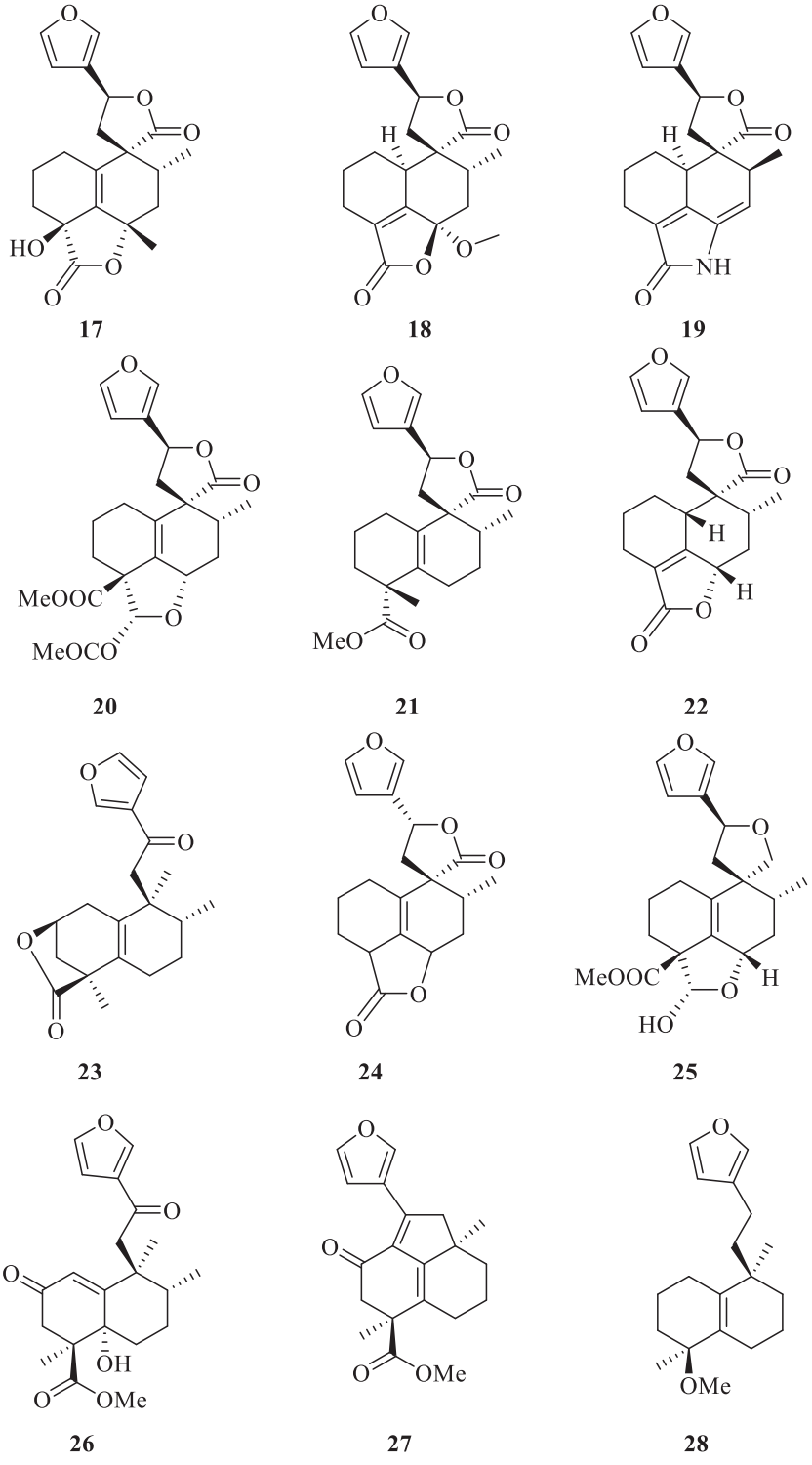

Fig. 2 Structures of diterpene compounds of *C. crassifolius*

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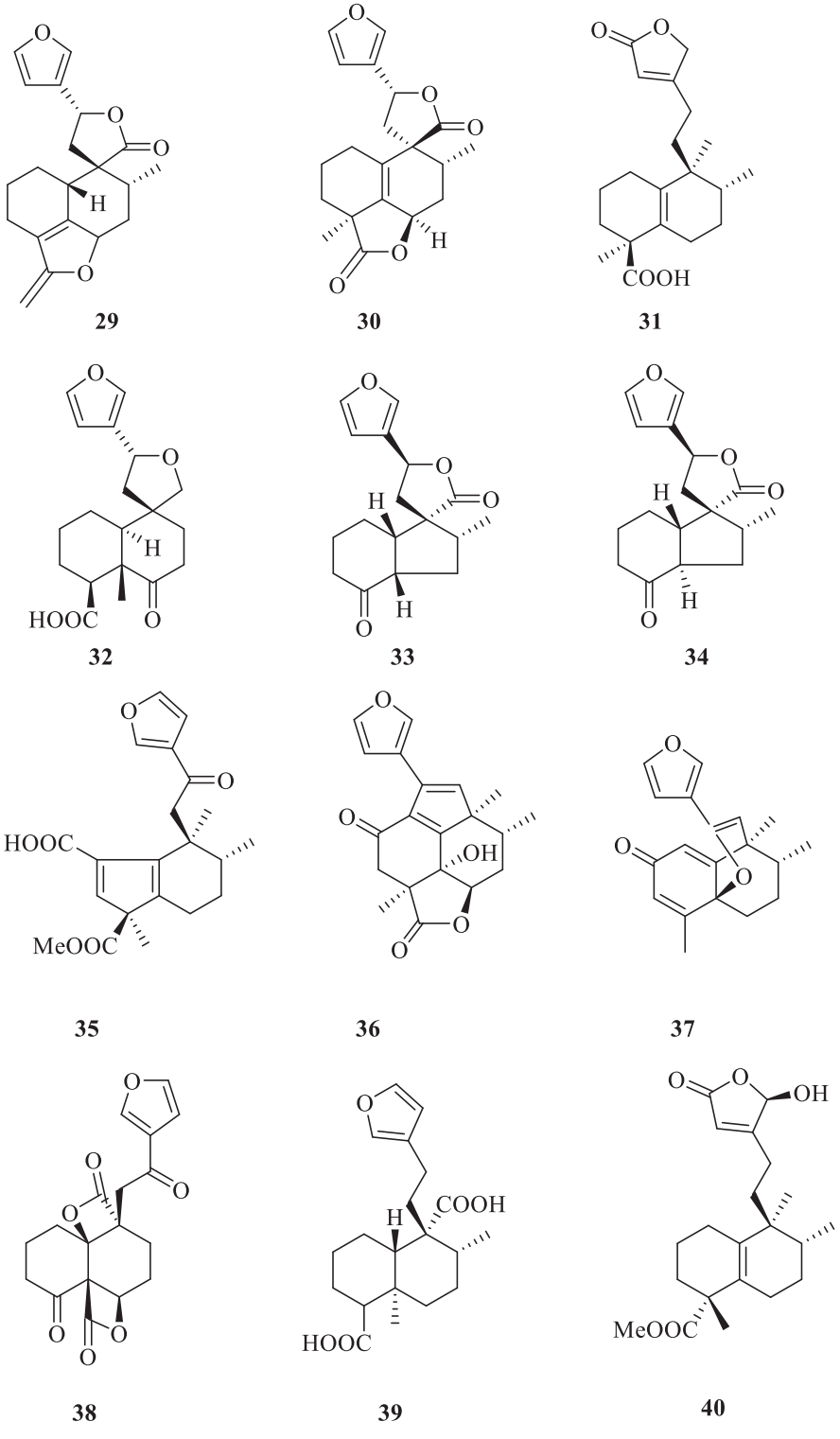

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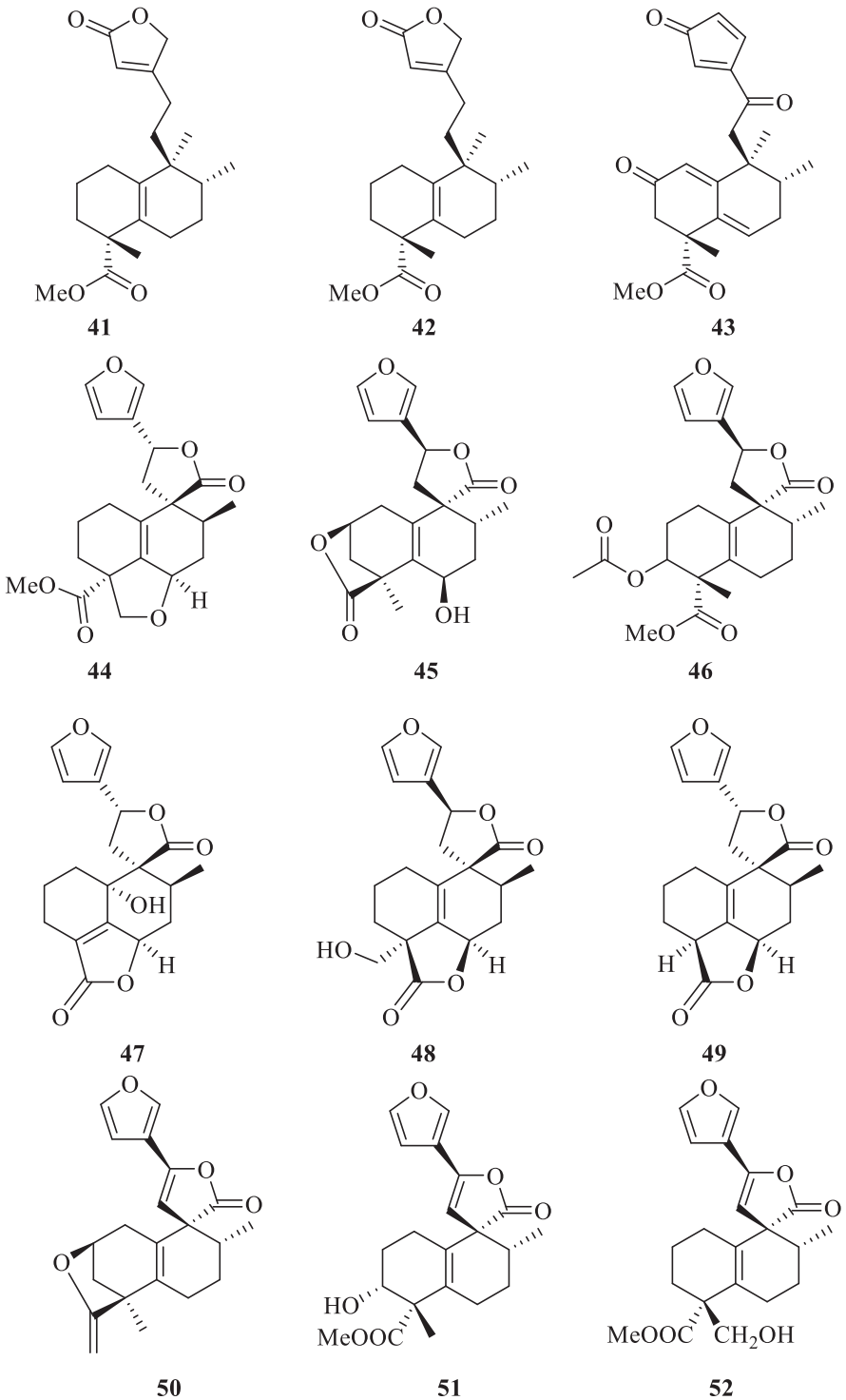

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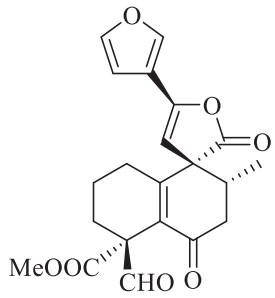

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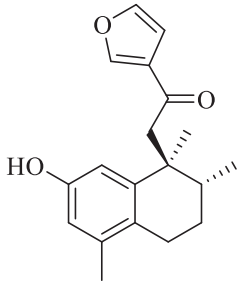

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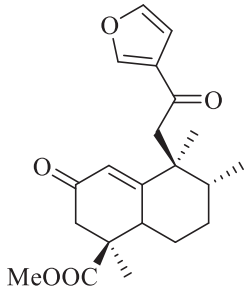

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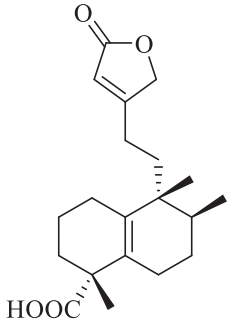

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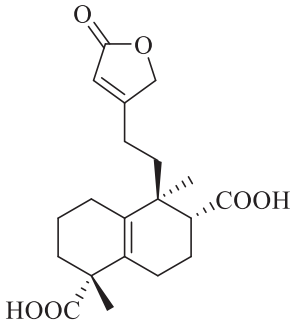

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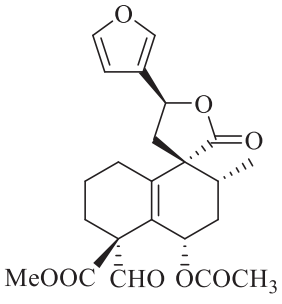

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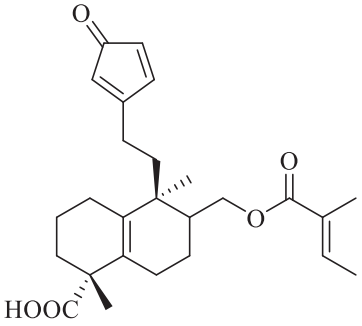

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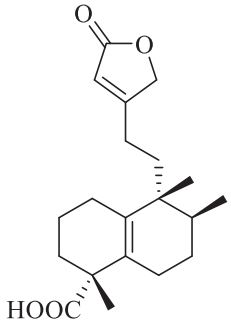

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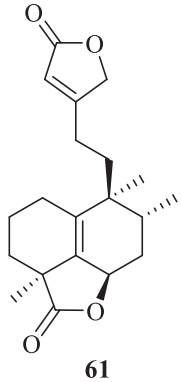

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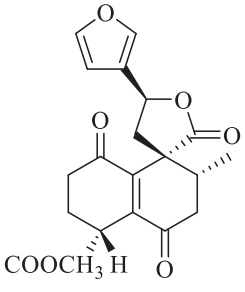

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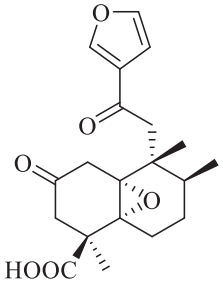

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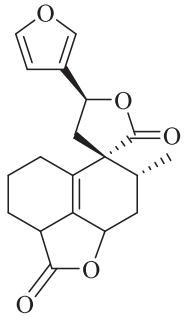

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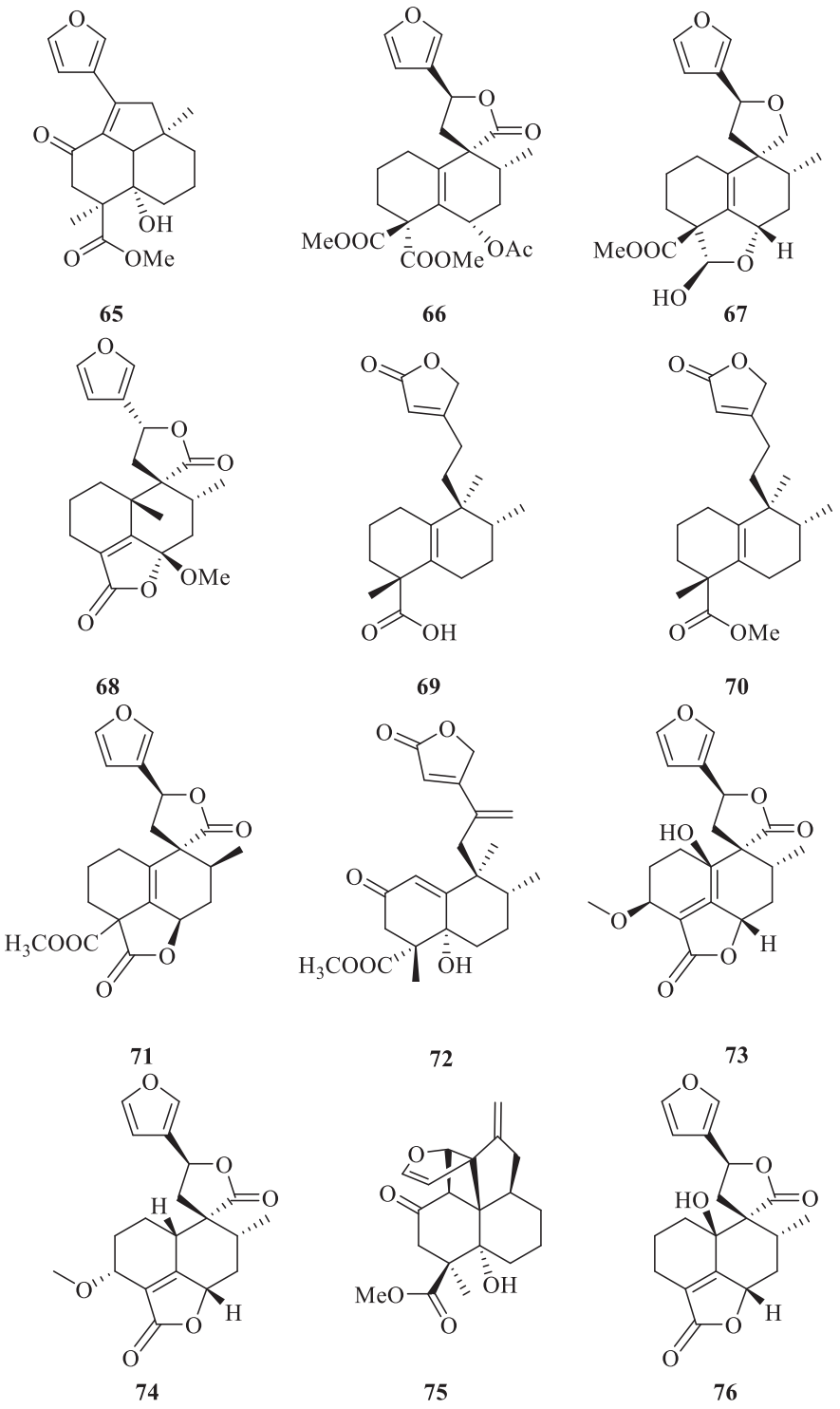

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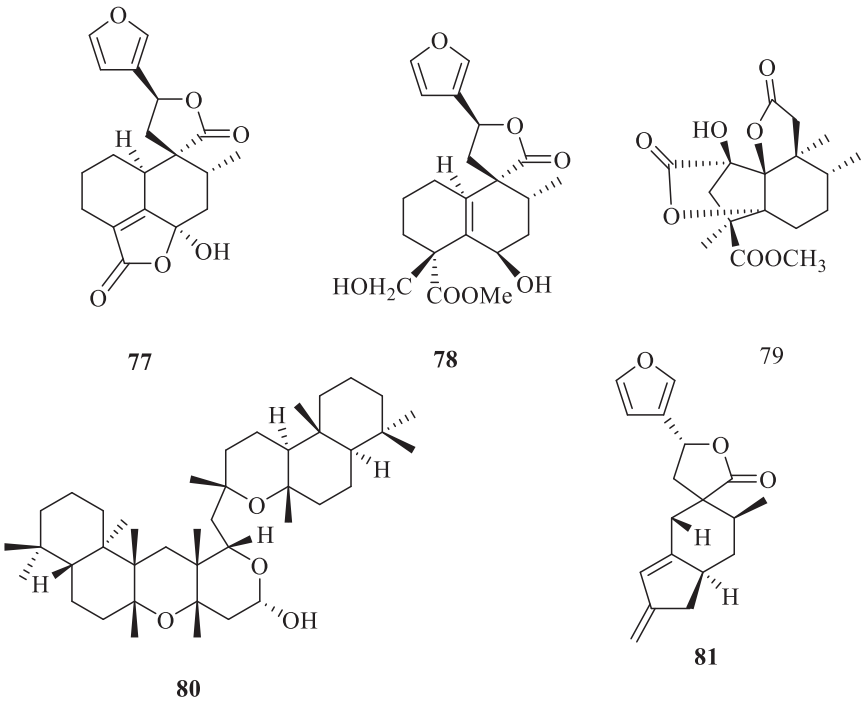

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2.1.3 Triterpenoid components

A few species of triterpenoids were isolated from *C. crassifolius*. Their structures are shown in Table 3 and Fig. 3.

Table 3 Triterpenoids in *C. crassifolius*

No.	Chemical component	References
82	lupeol	[3]
83	acetyl aleuritolic acid	[3]
84	lupenone	[3]
85	aleuritolic	[12]
86	epitaraxerrol	[12]
87	*β*-amyrin	[12]
88	aleuritolic acid	[13]

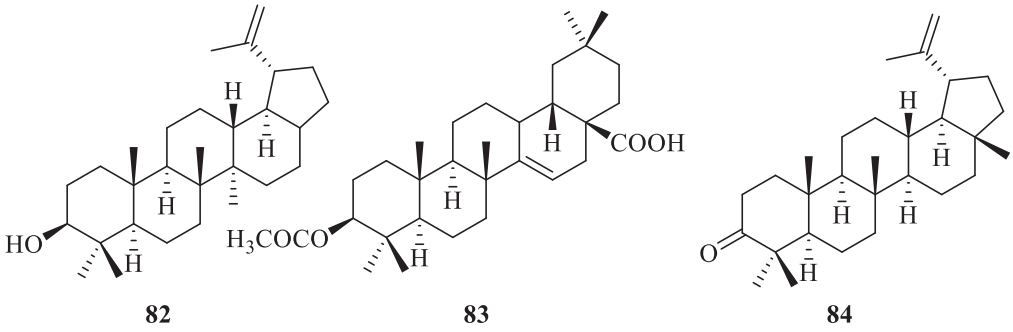

Fig. 3 Structures of triterpenoids in *C. crassifolius*

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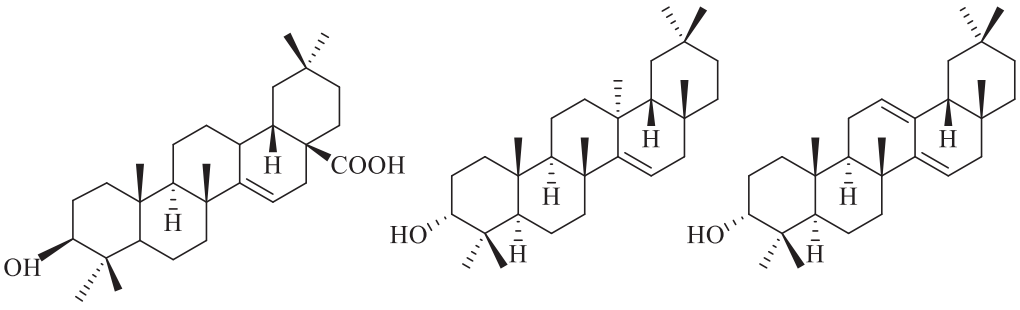


85 **86** **87**

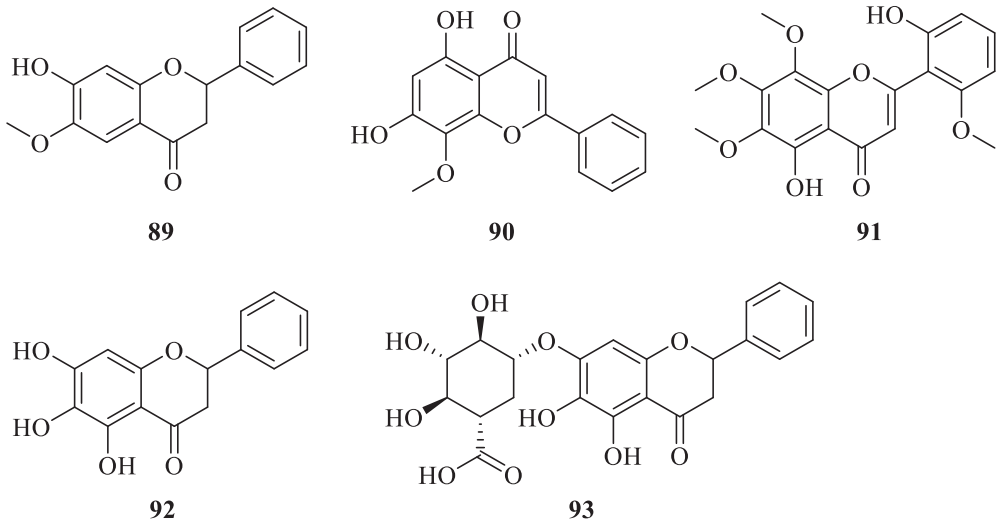
Continued fig. 3

2.2 Flavonoid composition

The flavonoid compounds of *C. crassifolius* are oroxylin A, wogonin, skallcapflavon II, baicalein and baicalin shown in Table 4 and Fig. 4.

Table 4 Flavonoids in *C. crassifolius*

No.	Chemical component	References
89	oroxylin A	[14]
90	wogonin	[14]
91	skallcapflavon II	[14]
92	baicalein	[14]
93	baicalin	[14]



89 **90** **91**

92 **93**

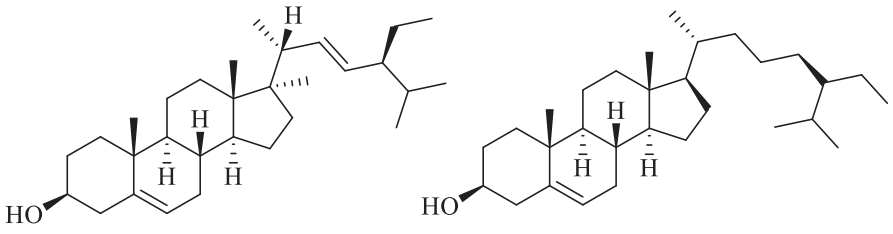
Fig. 4 Structures of flavonoids in *C. crassifolius*

2.3 Steroidal components

The steroidal components of *C. crassifolius* are stigmasterol and *β*-sitosterol. Their structures are shown in Table 5 and Fig. 5.

Table 5 Steroidal components in *C. crassifolius*

No.	Chemical component	References
94	stigmasterol	[15]
95	*β*-sitosterol	[15]



94 **95**

Fig. 5 Structures of steroidal components of *C. crassifolius*

3 Pharmacological activity of *C. crassifolius*

3.1 Antiviral activity

Researchers isolated a series of clerodane diterpenoids crassifolins A-G from *C. crassifolius*. Activity screening showed that the these diterpenoids had weak anti-Herpessimplex virus type I (HSV-1) viral activity (IC_{50} > 25 *μ*g/mL) [8].

3.2 Anti-cancer activity

Cancer is a malignant disease caused by the cancer of normal cells, and it is an important factor that damages human health in the 21st century. Liu et al. applied supercritical fluid extraction to obtain two new diterpenoids, namely crassifolius P and crassifolius Q, from *C. crassifolius*. The anti-proliferative activity of these two compounds was tested *in vitro* using A549, HepG2 and Hela tumor cell lines. The results showed that the two new compounds exhibited significant selectivity towards tumor cells with IC_{50} values ranging from 20.43 ± 1.18 *μ*M to 25.72 ± 1.32 *μ*M [9]. Qiu et al. evaluated the anticancer activity of terpenoids in *C. crassifolius* against cancer cell lines T24 and A549 by CCK-8 assay and showed that cracroson D and cracroson F had moderate anticancer activity [10]. The terpenoids and flavonoids in *C. crassifolius* have inhibitory effects on different cancer cell lineages through the induction of autophagy dysregulation, inhibition of angiogenesis, inhibition of cancer cell proliferation, promotion of apoptosis and promotion of cell pyroptosis. Researchers investigated the inhibitory effect and mechanism of aleuritolic acid, a triterpene isolated from *C. crassifolius*, on HepG2 with MTT method. They treated HepG2 cells with different concentrations of aleuritolic acid (100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0 *μ*M) for 24 h and found that aleuritolic acid caused autophagy dysregulation (enhanced conversion of LC3-I to LC3-II, p62 accumulation) in HepG2 cells, and caused autophagy dysregulation to produce toxic factors that promote cancer cell death. The team also found that inhibition of autophagosome production by knocking down ATG5 or using phosphatidylinositol 3-kinase inhibitors reduced the cytotoxicity of aleuritolic acid [13]. Researchers

utilized the supercritical extraction method to extract essential oil from Houttuynia cordata. They employed the CCK-8 assay to investigate its anti-proliferative effects on five types of tumor cells. Results showed that the essential oil of *C. crassifolius* had the best anti-proliferative activity against the cancer cell line A549 with the lowest IC_{50} values of 25.00±1.62 μM/mL, and further studies have shown that the essential oil extract of *C. crassifolius* can arrest the cell line A549 in the G2/M phase by decreasing the expression of cyclin B1-CDK1 and cyclin A-CDK1 and increasing the expression of cyclin-dependent kinase inhibitor (CKI) [16]. Dai et al. found that mallotucin D, a diterpene component of *C. crassifolius*, caused mitochondrial damage, decreased TOM20 expression and mitochondrial membrane potential, and induced excessive production of ROS. Mallotucin D can also promote the entry of cytochrome from mitochondria into the cytoplasm of hepatoma cells, resulting in the cleavage of cystein-9 and cystein-3, and the induction of GSDMD-associated pyroptosis in the hepatoma cell line HepG2 [17]. Tian et al. isolated a pyran-2-one derivative from *C. crassifolius* and found that it was toxic to hepatocellular carcinoma cells HepG2. Further studies demonstrated that this compound could inhibit Ras/Raf/ERK through p53-mediated apoptosis of Hep3B cells [18].

3.3 Treatment of ulcerative colitis

Shiqin et al. investigated the therapeutic effects of *C. crassifolius* extract on DSS-induced ulcerative colitis (UC) in C57BL/6J mice. The C57BL/6J mice were divided into control group, dextran sulfate sodium (DSS) groups, mesalazine (100 mg/kg) groups, and *C. crassifolius* extract (150 and 600 mg/kg) groups. Except for the control group, other mice were provided with 3% DSS dissolved in distilled water for 7 d. Measures such as weight changes, disease activity index (DAI), colon length, and the expression of inflammatory mediators iNOS and COX-2 in colon tissue were used as indicators to assess the effect. The results demonstrated that *C. crassifolius* reduced weight loss, blood in the stool and intestinal inflammation in UC mice. The metabolomic study showed that the extract affected 25 biomarkers, 16 of which were associated with its efficacy, and the metabolic pathway suggested that the mechanism of the treatment of UC by the extract was related to the regulation of lipids (linoleic acid, sphingolipids, a-linolenic acid and glycerophospholipid). The metabolic pathways suggest a mechanism related to the regulation of lipids (linoleic acid, sphingolipids, a-linolenic acid and glycerophospholipid) [19].

3.4 Anti-angiogenic activity

Anti-angiogenesis therapy is an effective anti-tumor treatment, which works by inhibiting the formation of blood vessels in tumor cells, thereby suppressing the uptake of oxygen and nutrients by tumor cells. VEGFR2 is one of the subtypes of the VEGFG receptor consisting of seven l g subunits, a transmembrane structural domain and a tyrosine kinase structural domain. Its induced signaling pathway can promote angiogenesis. Tumor cell angiogenesis is an important process for tumor cell growth as well as metastasis, and targeting VEGFR2 inhibition can inhibit tumor cell angiogenesis and thus inhibit cancer [20]. The most potent two are sesquiterpene cyperenoic acid and diterpene penduliflaworsin. In further studies, the researchers investigated the mechanism of action of cyperenoic acid and penduliflaworosin by west blotting, and found that penduliflaworosin inhibited VEGFR2 phosphorylation, while cyperenoic acid targeted VEGFR2 by inhibiting VEGFR2 tyrosine kinase activity [21,22]. Researchers extracted eight compounds from the *C. crassifolius*, and utilized an *in vitro* model of human umbilical vein endothelial

cells to determine the anti-angiogenic activity of compounds crassifolins Q-U. The results indicated that all compounds exhibited anti-angiogenic activity, with crassifolin U showing the strongest activity in the range of 6.25-50 μM [23,24].

3.5 Treatment of osteoporosis

Cyperenoic acid, a terpene compound of *C. crassifolius*, was found to reduce bone loss. Researchers found that cyperenoic acid reduced bone loss in SAMP6 mice, and further studies demonstrated that cyperenoic acid could inhibit the activation of the NF-κB pathway p100/p53 under RANKL stimulation and then repressed two key transcription factors in osteoclast differentiation, N FATc 1 and c-Fos. Cyperenoic acid also inhibited the expression of some osteoclast-related genes [25].

3.6 Anti-inflammatory activity

Li et al. found that crassifolin U, a diterpene compound of *C. crassifolius*, reduced the secretion levels of LPS-induced inflammatory factors IL-6 and TNF-α in PAW264.7 macrophages by 32.78% and 12.53%, respectively [26]. Researchers used methanol for extraction over a period of 7 d. The process involved silica gel column chromatography with elution using a mixture of petroleum ether and ethyl acetate (20:0-0:1). Subsequently, separation was carried out using an ODS column with elution using CH_3OH-H_2O (ranging from 3:7 to 1:0). Finally, purification was conducted through semi-preparative high-performance liquid chromatography on an ODS column. As a result, they obtained a set of isomers (3*S*-methoxyl-teucvin and 3*R*-methoxyl-teucvin). The anti-inflammatory activity of the diterpene compounds, 3*S*-methoxyl-teucvin and 3*R*-methoxyl-teucvin, from *C. crassifolius* was found by dexamethas one control experiments with IC_{50} values of 0.82 and 0.54 μM [27]. Chen et al. used leukocyte elastase inhibitors as high-throughput screening model to screen *C. crassifolius* antipyrotic compounds. They found chettaphanin I and penduliflaworosin had strong inhibitory activity against human leukocyte elastase (IC_{50} 8.49 and 36.0 μg/mL, respectively) [28]. The ethanolic extract of *C. crassifolius* reduced acetic acid-induced torsion and formalin-induced pain in mice, while the ethanolic extract of *C. crassifolius* reduced acetic acid-induced increase in capillary permeability and carrageenan-induced paw edema in mice [29].

3.7 Anti-Alzheimer activity

Alzheimer's disease (AD) is a neurodegenerative disease, and the most important pathological change in the brain of AD patients is the deposition of β-amyloid protein (Aβ). *Caenorhabditis elegans* model is a common animal model for studying AD, which can exhibit AD symptoms induced by Aβ neurotoxicity [30]. Norcrassin A and bicrotonol A are two diterpenoid compounds of *C. crassifolius*. Researchers used them in Alzheimer's model of *Caenorhabditis elegans* and showed that these two compounds had anti-AD activity [11].

4 Conclusion

Here we summarize all the studies on *C. crassifolius*. The chemical constituents of *C. crassifolius* include terpenoids, flavonoids and sterols. *C. crassifolius* is rich in terpenes, which possess the main biological activities, with diterpenes being the most abundant among them. The chemical constituents in *C. crassifolius* have shown activities in the treatment of many diseases. The most detailed studies on its pharmacology focus on its anti-tumor activity and its mechanism. In the future, researchers will isolate and identify new terpenoids from *C. crassifolius*, and components and pharmacological activities of *C. crassifolius* await further research.

Acknowledgements

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