



Review

Review on *Croton crassifolius*: From botanical characteristics to biotransformation and better utilization

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Abstract

Croton crassifolius (*C. crassifolius*) is a kind of traditional Chinese medicine, with a history hundreds of years in China. By consulting Scencedirect databases and Web of Science databases, 68 related articles were found, 12 of which were related to chemical composition and pharmacological activity. It was used by the ancients as the medicine to treat stomach trouble, rheumatism, poliomyelitis and injuries, and it is the treasure of traditional Chinese medicine in China. With the development of modern medicine, the active ingredients and therapeutic mechanism of *C. crassifolius* have been gradually revealed. In this study, the functions and applications of *C. crassifolius* were reviewed from the aspects of botany, traditional Chinese medicine therapy and biotransformation of substances, so as to better understand and use *C. crassifolius* and provide more effective drug choices for clinical treatment.

Keywords: *Croton crassifolius*; botany; biotransformation; utilization

1 Introduction

Euphorbiaceae is a large and diverse family consisting of approximately 300 genera. The genus *Croton* includes 1300 species and consists of a variety of trees and shrubs distributed all over the world. The genus plays a crucial role in Chinese

traditional medicine. *Croton crassifolius* is mainly found in the south and southeast of China, Thailand, Vietnam and Laos. It is a shrub plant, about 20-50 cm high, densely covered with stellate villi in all parts, with various leaf shapes, 3-5 basal veins and prominent pedunculated goblet glands. The inflorescence racemose is terminal, with different structures of male and female flowers, nearly spherical fruit and oval seeds, flowering from November to June [1]. In the field of traditional medicine, *C. crassifolius* is warm in root and bitter in taste, and is widely used for treatment. Bencao Qiuyuan emphasizes its efficacy in treating rheumatism, strengthening tendons and bones, and

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dispelling diseases. The Processing Standard of Chinese Herbal decoction Pieces of Guangxi Zhuang Autonomous Region further explains that it is slightly toxic, attributed to the meridians of stomach, large intestine and liver [2]. Its functions include regulating Qi and relieving pain, expelling wind and removing dampness, relaxing tendon and activating collaterals, and it is often used to treat stomach disease, rheumatism and traumatic injury.

2 Botany

2.1 Plant Morphology

C. crassifolius is a kind of shrub, 20-50 cm tall. The branches, young leaves, lower part of growing leaves, inflorescences and fruits are densely covered with stellate villi. The old branches are subglabrous. The leaves are ovate, ovoid oval to oblong, 4-10 cm long, 2-6 cm wide, the apex is obtuse to short pointed, the base is subrounded to microcordiform, the edge has inconspicuous fine teeth, and sometimes there are glands between the teeth. During the

growing period, the hairs on the leaves gradually fall off, the rest hairs are rough at the base and turn black after drying. Basal efferent veins are 3 (-5), and lateral veins are (3-) 4-5 pairs. The petiole is 2-4 cm long. Two pedunculated goblet glands are on both sides of the midvein at the base of the leaf blade or the apex of petiole. Stipules are drillate, 2-3 mm long, caducous. Raceme is terminal, 5-10 cm long. Bracts are linear, 2-4 mm long, with linear teeth at the edge and small capitate glands at the tip. Male plants have sepals outside stellate villi, the petals oblong, as long as sepals, and the edges are woolly. There are 14-20 stamens. Female plants have sepals outside stellate villi, the ovary is densely yellow villous, and the style is 4 deeply lobed and linear. The fruit is subspherical, about 1 cm in diameter, and the seeds are oval and brown, about 5 mm long. It flowers from November to June of the following year. Produced in Fujian, Guangdong, Guangxi, Hainan and other places, it grows in coastal hills and mountains rather than on dry slopes [1]. It is also distributed in Vietnam, Laos and Thailand (Fig. 1) .



Fig. 1 The appearance of *C. crassifolius*

2.2 Plant anatomy

The cross section of the plant root is round, its cork cells are arranged neatly in 10-15 rows, and the appearance is rectangular and reddish brown. The cortex contains round parenchymal cells, oil cells and calcium oxalate clusters. The fiber bundles in the columnar sheath are distributed in discontinuous annular lignified structures. The xylem is well-developed with tracheids either appearing alone or radially aligned in groups of 2-5, coexisting with calcium oxalate clusters and oil cells in the medulla.

In the nearly circular cross section of the stem, cork cells are composed of multiple layers of closely spaced rectangular tangential tissues that harbor reddish-brown substances. The cortex is made

up of various cell types, where the fiber bundles arranged intermittently in a ring to form a part of the endodermis layer [3].

In the leaf cross-sections, ordered parenchymal cells cover both epidermal layers with glands and stellate hairs. The palisade tissue consists of reddish-brown cells, spongy tissue and calcium oxalate crystals. Midrib epidermis features brown-red tissues and the inner epidermis is thickened. Mesophyll cells are small and dense, with oil cells, cambium and 1-3 radial xylem vessels. Vascular bundles contain two main veins, grooved and surrounded by fibers, large calcium oxalate clusters, and oil cells, plus 4-8 radial xylem vessels. The upper epidermal cells have irregular shapes, straight walls and visible stellate hairs [4] (Fig. 2).

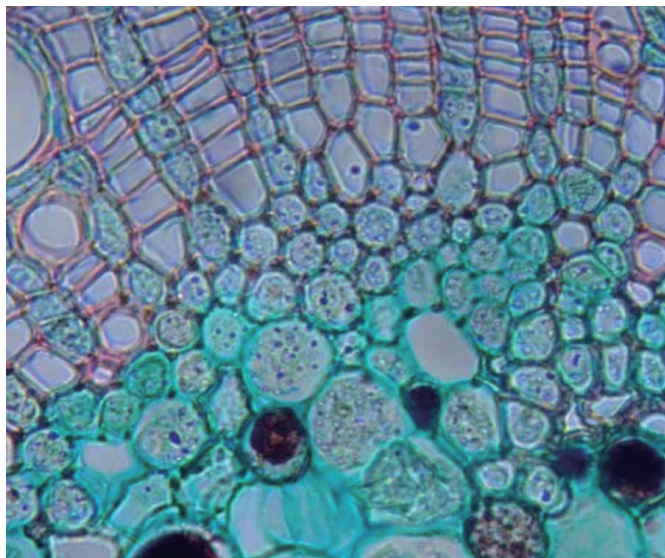


Fig. 2 Micron-level structure of fiber bundle

2.3 Plant genetics

A systematic study on the content of compounds in *C. crassifolius* has led the researchers to speculate about the potential genetic relationships among the genera *Euphorbia*, *Joannesia*, *Colchicum*, *Sapium* and *Croton*. Specifically, scientists have found that these species show remarkable consistency in metabolic pathways. In-depth analysis shows

that some compounds found in the land plant *C. crassifolius* are widely distributed, not only in *Asteraceae*, but also in a variety of taxa, such as *Pinaceae* and *Zea*. For example, the sources of some macrolide compounds range from *Lasiodiplodia* [5] to fungi [6] in Marine environments. It is noteworthy that some compounds have a wide range of families and genera and even cross-ecosystem phenomenon. For example, compounds from the Marine branch are also



present in the land plant *C. crassifolius*. This question urgently needs to be answered by further research in evolutionary development and genomics [7]. This extensive distribution pattern may reveal the conservation of compound biosynthetic pathways, convergent evolution or the influence mechanism of ecological interactions [8].

3 Traditional Chinese Medicine therapy

The application of *C. crassifolius* was recorded in two books, Sheng Cao Yao Xing Bei Yao (Essentials of Raw Herbs) and Lingnan Collected Medicine Record. Among them, Sheng Cao Yao Xing Bei Yao elaborates its pungent, bitter and warm properties, and clearly points out that it can be used to treat sore throat and heart qi pain. However, Bencao Qiyuan (prescriptions for Materia Medica) mentions that it has the effects of dispelling wind and dampness, strengthening tendons and bones, and dissipating diseases. In addition, detailed records of *C. crassifolius* were found in such authoritative Chinese medicine monobooks as Zhong Yao Da Qun Dian, the National Compendium of Chinese Medicinal Herbs and Chinese Materia Medica Cihai. According to the Processing Specification of Chinese Herbal decoction Pieces issued by Guangxi Zhuang Autonomous Region, it is defined as spicy and bitter, warm in nature and slightly toxic, which is mainly attributed to the three meridians of stomach, large intestine and liver. Its functions include but are not limited to regulating Qi and relieving pain, dispelling wind and removing dampness, and relaxing tendons and activating collaterals. It is widely used in clinical practice to relieve symptoms such as stomach pain, flatulence, rheumatism and pain, and falls and injuries.

C. crassifolius has played an important role in traditional Chinese medicine, used to treat a variety of diseases. For patients with cold RA, especially middle-aged and elderly people, joint pain is often

aggravated when the climate is cold and humid. *C. crassifolius*, combined with other herbs such as cinnamomum twig and Rhizoma pilosa, was used to regulate the meridians, relieve the sinew and activate the collaterals. Centipede and aconite were added to enhance the curative effect. In terms of the chronic disease of deficiency and cold stomach pain, it is manifested as pale complexion and stomach pain is often aggravated by cold. In this case, *C. crassifolius* are combined with pieces of Qiang Huang, dried ginger, galangal ginger isothermal dispersive cold and Qi and pain relief products, or, according to the different symptoms of patients, combined with add cuttlebone, raw panax notoginseng powder, Picrorhizoma coptidis, Sanguisorba and other medicinal materials to assist the treatment. In the treatment of joint muscle and tendon strain pain caused by long-term labor or excessive weight bearing, *C. crassifolius* is also a key component, together with other herbs such as peach kernel, Tianxiang kiln, Chuanxiong and Angelica sinensis, which promote blood circulation, dissipate blood pressure, and relieve pain, and according to the location and symptom characteristics of the disease, Qiang Huo, Phellodendron, Lonicerae and liquor can be added as appropriate to promote the operation of medicine or heat and detoxification [9].

4 Biotransformation

4.1 Research progress of biotransformation

Biotransformation is a technology that uses an enzyme or a series of enzymes produced in the process of metabolism in the organism to modify the chemical structure of a specific part of the substrate. A variety of biocatalases are used in this technology, which can cause various types of reactions of the added substrates, such as REDOX reaction, de-racemization reaction, hydrolysis reaction, glycosylation reaction, methylation/demethylation

reaction, etc. With the deepening of microbial diversity research, especially the developments of genomics, proteomics and metabolic engineering technologies, the application fields of biotransformation technology have been further expanded. Biotransformation plays an increasingly important role in the structural modification of active ingredients of natural products [10].

Microbial transformation technology is of great value in the development of traditional Chinese medicine. Microbial cell factories are an effective way to solve the scarcity problem of plant-derived natural compounds, such as ginsenosides and taxol [11]. It is known that microbial transformation involves a variety of chemical reaction types, and one or more reactions may be involved in the transformation process of a TCM. Among them, hydroxylation is the most common and important reaction, which can produce a variety of hydroxylated derivatives. The active ingredients of traditional Chinese medicine generally include glycosides (terpenoids, steroids), flavonoids, phenylpropanoids, alkaloids, quinones, tannins, esters, etc. In general, obtaining different active ingredients involves different types of reactions.

Compared with traditional physical and

chemical methods, microbial transformation of Chinese herbal medicine can change the properties to the greatest extent and improve the efficacy. Yu et al. performed microbial transformation of Rb1, Re and Rg1 in ginseng, and the yield of the products and the anti-inflammatory effect were significantly improved ($P < 0.05$) [12].

4.2 Biotransformation pathway of Methyl cyperenoate

Cunninghamella elegans AS 3.2028 was applied to the sesquiterpenoid compounds Cyperenoic acid (JGX-27) and Methyl cyperenoate (JGX-29) isolated from *C. crassifolius*. The biotransformation products of Methyl cyperenoate were isolated by extraction-open ODS column chromatography and reversed-phase preparation of liquid phase, six of which were obtained by hydroxylation, including 9*S*-hydroxy-methyl cyperenoate (1), 8*R*-hydroxy-methyl cyperenoate (2), 8*S*-hydroxy-methyl cyperenoate (3), 13*R*-hydroxy-methyl cyperenoate (4), 10*S*-hydroxy-methyl cyperenoate (5) and 7*S*-hydroxy-methyl cyperenoate (6). The structures of the products obtained after hydroxylation by JGX-29 are shown in Fig. 3.

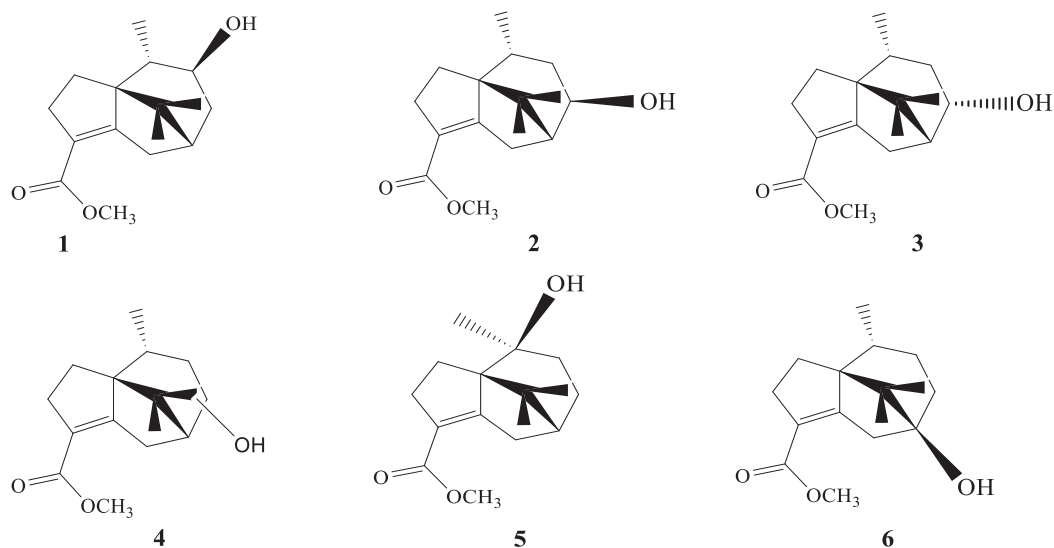


Fig. 3 Structures of the products obtained after hydroxylation by JGX-29



In modern cosmetics, traditional Chinese medicine can be used as an effective additive, providing nutrition and efficacy for cosmetics, improving skin texture, whitening skin, diminishing inflammation, protecting skin, resisting corrosion and oxidation, emulsifying, perfuming and toning [14].

In order to efficiently utilize resources and protect the environment, the comprehensive utilization of pharmaceutical residues mainly focuses on the following aspects: firstly, through biotechnology means, such as microbial fermentation, the pharmaceutical residues are transformed into high-protein feed, which significantly improves its economic value; secondly, the active ingredients with potential utilization, such as polysaccharides, are extracted from the residue by enzymatic hydrolysis. In addition, pharmaceutical residues can be used as raw materials to produce alcohol or processed into organic fertilizer, which can be mixed with poultry manure and used for the cultivation of medicinal plants to form an ecological recycling mode [15].

5.2 Other Applications

Network pharmacology is helpful to study the characteristics of multi-component, multi-target and multi-pathway synergistic effects of traditional Chinese medicine. By constructing the “drug-target-disease” network, we can systematically explain the mechanism of action of traditional Chinese medicine compound at the molecular level, so as to make full use of traditional Chinese medicine. Network pharmacology can find the effective components and targets of traditional Chinese medicine by screening its component library, and predict the mechanism and efficacy of traditional Chinese medicine. This will help promote the modernization and internationalization of traditional Chinese medicine [16].

Although chemical composition analysis and pharmacological studies have been carried out on

C. crassifolius, there is a lack of data and studies to evaluate its toxicity and pharmacokinetics. Whether microbial transformation of the active ingredients of *C. crassifolius* can be applied to actual production and clinical effect is also unknown.

6 Conclusion

This article reviews the botanical research of *C. crassifolius*, the application of traditional Chinese medicine and its biotransformation and utilization. These studies are of great significance for in-depth understanding of the mechanism of action of *C. crassifolius* and its active components, and can provide more effective drug selections for clinical treatment. However, at present, there are few studies on the biotransformation of the chemical components of *C. crassifolius*. In addition, it is necessary to further evaluate its toxicity and side effects and improve the laboratory preparation methods.

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References

- [1] Qiu MS, Cao D, Gao YH, et al. New clerodane diterpenoids from *Croton crassifolius*. *Fitoterapia*, 2016, 108: 81-86.
- [2] Sun M, Zeng W, Zhao Z. *Croton crassifolius* Geisel: A comprehensive review of the botany, traditional uses phytochemistry, pharmacology, and quality control. *J Ethnopharmacol*, 2023, 3: 117352-117355.
- [3] Costa R, Lourenço A, Oliveira V, et al. Chemical characterization of cork, phloem and wood from different *Quercus suber* provenances and trees. *Heliyon*,



- 2019, 5: 2-5.
- [4] Huang JZ, Weng WF. Pharmacognostical study of *Croton Crassifolius Geisel*. J Holist Integr Pharm, 2023, 4: 234-240.
- [5] Huang JG, Xu JY, Wang Z, et al. New lasiodiplodins from mangrove endophytic fungus *Lasiodiplodia* sp. 318#. Nat Prod Res, 2017, 31: 326-332.
- [6] Umeokoli, Ebrahim W, El-Neketi M, et al. A new depsidone derivative from mangrove sediment derived fungus *Lasiodiplodia theobromae*. Nat Prod Res, 2019, 33: 2215-2222.
- [7] Wei XP, Li HJ, Che P, et al. Comparing chloroplast genomes of traditional Chinese herbs *Schisandra sphenanthera* and *S. chinensis*. Chin Herb Med, 2020, 12: 247-256.
- [8] Li JY, Bai M, Song SJ, et al. Chemical constituents from the roots of *Croton crassifolius* and their chemophenetic significance. Biochem Syst Ecol, 2023, 107: 1-3.
- [9] Pan WZ. *Croton Crassifolius* can treat strain joint muscle pain. New Rural Tech, 2013, 1: 46-47.
- [10] Chen Y, Tian JL, Wu JS, et al. Biotransformation of cyperenoic acid by *Cunninghamella elegans* AS 3.2028 and the potent anti-angiogenic activities of its metabolites. Fitoterapia, 2017, 118: 32-37.
- [11] Jiang FL, Gong T, Chen JJ, et al. Synthetic biology of plants-derived medicinal natural products. Chin J Biotech, 2021, 37: 1931-1951.
- [12] Guan SL, Wu YX, Sun H, et al. Application of microbial transformation technology in the development of Chinese medicine. Microb Chin, 2018, 45: 900-906.
- [13] Tian JL, Chen Y, Wang TX, et al. Microbial transformation of methyl cyperenoate by *Cunninghamella elegans* AS 3.2028 and the antithrombotic activities of its metabolites. RSC Advances, 2016, 6: 112712-112720.
- [14] Huang FL. The application of traditional Chinese medicine in modern cosmetics. J Tradit Chin Med, 2001, 2: 48-50.
- [15] Wu CJ, Wang YT, Lei PL. Comprehensive utilization and treatment of traditional Chinese medicine residue. China J Chin Mater Med, 1998, 1: 59-60.
- [16] Li X, Liu Z, Liao J, et al. Network pharmacology approaches for research of Traditional Chinese Medicines. Chin J Nat Med, 2023, 21: 323-332.