# Research Progress on Chemical Components of Trichosanthis Fructus

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**Abstract** Trichosanthis Fructus (Gualou) is the dried ripe fruit of *Trichosanthes kirilowii* Maxim. or *Trichosanthes rosthornii* Harms (Cucurbitaceae family). It demonstrates significant therapeutic effects in treating coronary heart disease and diabetes mellitus, while also possessing anti-cancer and anti-aging properties. Both Trichosanthis Pericarpium (Gualoupi) and Trichosanthis Semen (Gualouzi) are used medicinally. With the increasing clinical application of Trichosanthes medicinal materials and their preparations (such as Gualou Injection and Gualou Tablets), the demand for them is expected to grow substantially. To further investigate Trichosanthis Fructus, explore its medicinal effects and new applications, and promote the development of Trichosanthis Fructus-based products, this paper reviewed the chemical components of Trichosanthis Fructus. Furthermore, the differences in major chemical components among Trichosanthis Fructus, Trichosanthis Pericarpium and Trichosanthis Semen were compared, providing a scientific basis for establishing quality evaluation standards of *Trichosanthes* medicinal materials.

**Key words** Trichosanthis Fructus; Trichosanthis Pericarpium; Trichosanthis Semen; Chemical components

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Trichosanthis Fructus (Gualou) is the dried ripe fruit of *Trichosanthes kirilowii* Maxim. or *Trichosanthes rosthornii* Harms from the Cucurbitaceae family, also known as "Quangualou"[1]. It is sweet and slightly bitter in taste and cold in nature, and attributive to the lung, stomach, and large intestine meridians. Trichosanthis Fructus has the effect of clearing away heat, removing phlegm, relieving chest pain, dispersing stagnation, moistening dryness and smoothing intestines, and is primarily used to treat lung-heat cough, thoracic obstruction, chest binding, and diabetes. It demonstrates significant efficacy in treating coronary heart disease, diabetes and tracheitis, and also has anti-cancer and anti-aging functions[2]. Both Trichosanthis Pericarpium (Gualoupi) and Trichosanthis Semen (Gualouzi) are used medicinally[1]. In recent years, scholars both at home and abroad have conducted extensive studies on the chemical constituents of Trichosanthis Fructus. Through comprehensive application of various extraction and isolation methods combined with spectroscopic techniques, nearly a hundred compounds have been identified. These mainly include bioactive substances such as terpenoids and their glycosides, flavonoids and their glycosides, sterols, saccharides, phenylpropanoids, alkaloids, oils, organic acids, proteins, amino acids, and trace elements[3-6]. This paper systematically summarized the chemical components of Trichosanthis Fructus, providing a basis for further research on its active components, exploration of new clinical applications, and development of novel drugs.

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## Terpenoids and Their Glycosides

Trichosanthis Fructus contains a large number of terpenoid compounds, primarily belonging to the triterpenoid class. Japanese scholar Akihisa Toshihiro *et al.*[7-12] isolated a series of triterpenoid compounds from Trichosanthis Semen, including karounidiol (1), 3-epikarounidiol (2), karounidiol-3-O-benzoate (3), 7-oxodihydrokarounidiol (4), 7-oxoisomultiflorenol (5), 5-dehydrokarounidiol (6), isokarounidiol (7), 7-oxo-8β-D: C-friedoolean-9(11)-ene-3α, 29-diol (8), 3-epibryonolol (9), bryonolol (10), 2, 4-dihydro-10α-cucurbitadienol (11), 7-oxo-10α-cucurbitadienol (12), and 7-oxo-D: C-isooleana-8-ene-3β-ol (13). Kimura Yumiko *et al.*[13] isolated two new cycloartane-type triterpenoids from Trichosanthis Semen, named cyclokirilodiol (14) and isocyclokirilodiol (15). Chinese scholars Wu *et al.*[14] obtained two new triterpenoid compounds from the chloroform extract of Trichosanthis Semen: (3α)-7-oxomultiflor-8-ene-3, 29-dihydroxy-3, 29-diyl dibenzoate (16) and (3α, 7β)-8-ene-3, 7, 29-trihydroxymultiflor-3, 29-diyl dibenzoate (17). Cheng *et al.*[15] isolated a new compound, 3, 29-dibenzoylkarounitriol (18), from Trichosanthis Semen. Tang[16] obtained a new triterpenoid from the unsaponifiable fraction of Trichosanthis Semen, named 6-hydroxydihydrokarounidiol, which was structurally identified as (3α, 6α, 13α, 14β, 20α)-3, 6, 29-trihydroxy-13-methyl-26-norolean-8-ene (19). Chao *et al.*[17-18] isolated two new triterpenoids from the unsaponifiable fraction of Trichosanthis Semen, named 7-oxodihydrokarounitriol and 7, 11-dioxodihydrokarounidiol. Their structures were identified as (3α, 11β, 13α, 14β, 20α)-3, 11, 29-trihydroxy-13-methyl-26-norolean-8-en-7-one (20) and (3α, 13α, 14β, 20α)-3, 29-dihydroxy-13-methyl-26-norolean-8-ene-7, 11-dione (21), respectively. Ma *et al.*[19] isolated a new pentacyclic triterpenoid, (3α, 5α, 8α, 20α)-5, 8-epidioxymultiflora-6, 9(11)-diene-3, 29-diol 3, 29-dibenzoate (22), and a triterpenoid compound, karouni-3, 29-diol 3, 29-dibenzoate (23), which was obtained from the *Trichosanthes* genus for the first time. Ma *et al*. [20] also isolated a new compound from prepared Trichosanthis Fructus cream, named 3, 29-dibenzoyloxykarounidiol (24). Additionally, Wei *et al*. [21] isolated a monoterpenoid compound, (-)-loliolide (25), from the lipophilic fraction of Trichosanthis Fructus grown in Shandong. Xu *et al*. [22] obtained two triterpenoid compounds from Trichosanthis Fructus, namely arvenins III (26) and opercurins A (27). Li *et al*. [23] applied ultra-high performance liquid chromatography-linear ion trap-Orbitrap high-resolution mass spectrometry (UPLC-LTQ-Orbitrap-MS) to identify several terpenoids in Trichosanthis Fructus, including the monoterpenoids 6, 9, 11-trihydroxy-4, 7-megastigmadien-3-one-11-O-β-D-glucopyranoside, 6, 9-dihydroxy-4, 7-megastigmadien-3-one-9-O-[α-L-apifuranosyl-(1→2)-β-D-glucopyranoside], 3, 5-dihydroxy-6, 7-megastigmadien-9-one, and 3-hydroxy-5, 6-epoxy-7-megastigmadien-9-one, as well as the triterpenoids cucurbitacin B, cucurbitacin D, and 23, 24-dihydrocucurbitacin B.

## Flavonoids and Their Glycosides

Although flavonoids are not characteristic components of Cucurbitaceae plants, scholars at home and abroad have successively isolated a series of flavonoids from Trichosanthis Fructus, Trichosanthis Pericarpium and Trichosanthis Semen. Dat Nguyen Tien *et al*. [24] isolated an extremely rare isoaurone-type compound from Trichosanthis Semen, structurally identified as 4′, 6-dihydroxy-4-methoxyisoaurone (28). Rahman Md. Aziz Abdur *et al*. [25] isolated several flavonoids from Trichosanthis Semen, including 2-(4-hydroxy-3-methoxyphenyl)-3-(2-hydroxy-5-methoxyphenyl)-3-oxo-1-propanol (29), 5, 7, 2′, 4′-tetrahydroxy-5′-methoxyflavone (30), and 7-hydroxy-4-benzopyrone (31). Japanese scholar Arisawa Munehisa *et al*. [26] obtained two flavonoid compounds from Trichosanthis Semen: 11-methoxy-nor-yangonin (32) and tricin (33). Chinese scholar Liu *et al*. [27] isolated diosmetin-7-O-β-D-glucoside (34) from Trichosanthis Fructus. Fan[28] obtained chrysoeriol-7-O-β-D-glucoside (35) from the n-butanol extract of 90% ethanol extract of Trichosanthis Fructus. Sun *et al*. [5] isolated chrysoeriol (36) and 4′-hydroxywogonin (37) from Trichosanthis Fructus. Xu[22] isolated 5, 6, 7, 8-pentamethoxyflavone (38) and 5, 6, 7, 8, 3′, 4′-hexamethoxyflavone (39) from Trichosanthis Fructus. Xu[29] obtained quercetin-3-O-α-riboside (40), quercetin-3-O-β-D-glucoside (41), luteolin-7-O-β-D-glucoside (42), and rutin (43) from Trichosanthis Pericarpium. Li[30] isolated several flavonoids from Trichosanthis Pericarpium, including quercetin-3-O-[α-L-rhamnose-(1→2)-β-D-glucopyranosyl]-5-O-β-D-glucopyranoside (44), apigenin-7-O-β-D-glucopyranoside (45), luteolin (46), apigenin (47), and diosmetin (48). An[31] obtained chrysoeriol-7-O-glucoside (49) from Trichosanthis Fructus. Recent studies have shown that Qiao *et al*. [32] detected flavonoid compounds including isoquercitrin and kaempferol in Trichosanthis Pericarpium using high-performance liquid chromatography (HPLC). Li *et al*. [23] identified quercetin and quercitrin in Trichosanthis Fructus using ultra-high performance liquid chromatography-linear ion trap-Orbitrap high-resolution mass spectrometry (UPLC-LTQ-Orbitrap-MS).

## Sterols

Studies have found that Trichosanthis Fructus, particularly Trichosanthis Semen, contains abundant sterol compounds, primarily including campesterol, stigmasterol, and sitosterol structures. They give the seeds their characteristic of "oil-rich" property. Both scholars at home and abroad have reported extensively on these compounds. Japanese scholar Kimura Yumiko isolated a series of dihydroxysterols from Trichosanthis Semen, structurally identified as stigmast-5-ene-3β, 4β-diol (50), stigmast-3β, 6α-diol (51), poriferast-3β, 6α-diol (52), poriferast-5, 25-diene-3β, 4β-diol (53), and poriferast-5-ene-3β, 4β-diol (54)[13]. Chinese scholar Chao[33-34] isolated a series of stigmasterol compounds from both Trichosanthis Pericarpium and Trichosanthis Semen. These were structurally identified as $\triangle^7$-stigmastenol (55), $\triangle^7$-stigmasten-3-one (56), $\triangle^7$-stigmastenol-3-β-D-glucopyranoside (57), β-sitosterol (58), stigmasta-7, 22-diene-3-O-β-D-glucoside (59), and stigmasta-7, 22-dien-3β-ol (60).

## Carbohydrate Compounds

Trichosanthis Fructus possesses a caramel-like aroma and tastes slightly sour and sweet. Traditional experience considers samples with "strong sweet flavor" to be of superior quality, indicating the presence of abundant carbohydrate components. Lian *et al*. [35] isolated two carbohydrate compounds from Trichosanthis Fructus, structurally identified as: 1, 3-O-[5-(hydroxymethyl)-furan-2-yl]methenyl-2-n-butyl-α-fructofuranoside (61) and n-butyl-3, 4-dihydroxyl-5-hydroxymethyl-4-O-[5-(hydroxymethyl)-furan-2-yl]-tetrahydrofuran-2-carboxylate (62). Chao *et al*. [34] isolated galactonic acid γ-lactone (63) and galactose (64) from the methanol extract of Trichosanthis Fructus. Shi[36] obtained glucose (65) from the n-butanol fraction of the ethanol extract of Trichosanthis Fructus. Fan[37] isolated six glycoside compounds from Trichosanthis Fructus, identified as: methyl-β-D-fructopyranoside (66), ethyl-β-D-fructopyranoside (67), n-butyl-β-D-fructopyranoside (68), ethyl-β-D-glucofuranoside (69), n-butyl-α-D-fructofuranoside (70), and n-butyl-β-D-fructofuranoside (71).

Chinese scholars have conducted extensive research on polysaccharide content determination in Trichosanthis Fructus. Yan[38] measured the reducing sugar and total sugar contents in unsteamed Trichosanthis Fructus as 5.54% and 6.71%, respectively, while the steamed samples showed 8.07% and 11.51% respectively. Significant differences were observed in both sugar contents before and after steaming. Sun[39] collected 18 batches of Trichosanthis Fructus decoction pieces from 15 provinces and cities. The study measured reducing sugar content ranging from 3.29% to 13.29% and total sugar content ranging from 16.17% to 32.65% in the samples, and proposed that high-quality Trichosanthis Fructus should contain more than 25% total sugar and over 9% reducing sugar. Qin[40] collected 15 batches of cultivated Trichosanthis

Fructus from Shandong Province, and measured their soluble polysaccharide contents ranging from 10.59% to 37.20% and total sugar contents ranging from 12.97% to 42.57%. Tu *et al.* [41] extracted polysaccharides from Trichosanthis Fructus by the water extraction and alcohol precipitation method through deproteinization and dialysis. After dextran gel column chromatography, thin-layer chromatography, and gas chromatography analysis, they determined that the polysaccharides mainly consist of glucose, arabinose, and rhamnose.

## Phenylpropanoid Compounds

Trichosanthis Fructus contains small amounts of phenylpropanoid compounds. Dat Nguyen Tien *et al.* [24] isolated one phenylpropanoid compound from Trichosanthis Semen, structurally identified as 6-(3-hydroxy-4-methoxystyryl)-4-methoxy-2H-pyran-2-one (72). Moon Surk-Sik *et al.* [42] isolated a series of lignan compounds from Trichosanthis Semen, and their structures were identified as: (-)-1-O-feruloylsecoisolariciresinol (73), (-)-secoisolariciresinol (74), 1, 4-O-diferuloylsecoisolariciresinol (75), (-)-pinoresinol (76), and 4-ketopinoresinol (77).

## Alkaloids and Nitrogen-containing Compounds

Trichosanthis Fructus contains trace amounts of alkaloid compounds. Chinese scholar Chao[43] isolated a novel alkaloid named trichosanatine (78) from the petroleum ether fraction of 70% ethanol extract of Trichosanthis Pericarpium. Its structure was identified as: α-benzamido-phenylpropionic acid 3-[(1-phenyl)ethylidene]amino-2-hydroxypropyl ester. Fan[28] isolated a novel alkaloid from the n-butanol fraction of 90% ethanol extract of Trichosanthis Fructus, structurally identified as: (2, 2′-bioxazolidine)-3, 3′-diethanol (79). Sun[5] obtained a new alkaloid from the 95% ethanol extract of Trichosanthis Fructus, which was structurally characterized as: 1-carboxypropyl-5-ethoxymethyl-1H-pyrrole-2-carbaldehyde (80). Liu[27] isolated several nitrogen-containing compounds from the 60% ethanol extract of Trichosanthis Fructus, including N-phenylphthalimide (81), 4-hydroxy-nicotinic acid (82), and adenosine (83). Xu isolated two alkaloid compounds from the 90% ethanol extract of Trichosanthis Fructus, identified as pyroglutamic acid (84) and ethyl pyroglutamate (85). Li[30] obtained several nitrogen-containing components from the water-soluble fraction of Trichosanthis Pericarpium, including cytosine (86), hypoxanthine (87), guanine (88), xanthine (89), guanosine (90), 6-isoinosine (91), and adenine ribonucleoside (92). Shi[36] isolated uracil from Trichosanthis Fructus. Zhang *et al.* [44] analyzed the chemical components in Guoloupi Injection using hydrophilic interaction liquid chromatography coupled with a quadrupole-Orbitrap-linear ion trap three-in-one high-resolution mass spectrometer. Through Compound Discoverer software analysis and database searches (ChemSpider and mzCloud), they identified 48 chemical constituents, including 24 alkaloids. The predominant alkaloids included trigonelline, stachydrine, and betaine.

## Lipids and Organic Acid Compounds

As a seed-based medicinal material, Trichosanthis Semen is rich in oils. Zeng *et al.* [45] extracted oil from Trichosanthis Semen and conducted gas chromatography-mass spectrometry (GC-MS) analysis. The results showed that the fatty acids in the seed oil mainly consist of unsaturated fatty acids, including lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and trichosanic acid. Yan *et al.* [46] conducted methyl esterification of *Trichosanthes* seed oil and performed GC-MS analysis. By combining MIST Search database retrieval and reference literature, they identified 19 fatty acids. Among these, nine unsaturated fatty acids (primarily linoleic acid, oleic acid, and trichosanic acid) accounted for 91.603% of the total content, while ten saturated fatty acids (mainly palmitic acid and stearic acid) constituted 8.019%. Chao *et al.* [33] isolated various organic acids from Trichosanthis Pericarpium, including tetracosanoic acid, cerotic acid, montanic acid, and mead acid. Chao[47] extracted volatile oils from Trichosanthis Pericarpium using steam distillation, prepared methyl ester derivatives, and identified 15 long-chain fatty acids including linolenic acid and n-pentadecanoic acid through GC-MS analysis. Chao[48] also analyzed the neutral fraction of Trichosanthis Pericarpium volatile oil by GC-MS, identifying 61 compounds accounting for 80.17% of the total neutral fraction. The five major components were dibutyl phthalate, methyl palmitate, phenanthrene, fluoranthene, and 3-methylphenanthrene. Joh Yong Goe analyzed the fatty oils in Trichosanthis Semen using GC-MS and identified major components including cis, cis-octadecadienoic acid, punicic acid, 9-cis-octadecenoic acid, α-eleostearic acid, and catalpic acid[49].

## Amino Acids and Trace Elements

Trichosanthis Semen contains abundant amino acids and trace elements. Chinese scholar Gong *et al.* conducted research on the amino acids in Trichosanthis Semen (divided into peel and peeled parts). Using an amino acid analyzer, they determined that both parts contained seven essential amino acids for humans (threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine) and ten non-essential amino acids (serine, glutamic acid, proline, glycine, alanine, cysteine, tyrosine, histidine, arginine, and aspartic acid). They also used atomic absorption spectrophotometry to determine trace elements in Trichosanthis Fructus (divided into peel and peeled parts). The results showed that both parts contained potassium, calcium, sodium, magnesium, iron, copper, zinc, manganese, cobalt, and nickel, while strontium was not detected in the peeled part[50]. Building upon these trace element measurements, Chao *et al.* [51] further determined the contents of aluminum, titanium, boron, chromium, and barium in Trichosanthis Pericarpium. Zhang *et al.* [52] analyzed the composition and content of amino acids in *Trichosanthes* peel, pulp, and seeds using UHPLC-MS/MS. The results showed that all three parts contained 19 amino acids (leucine, phenylalanine, γ-aminobutyric acid, tryptophan, isoleucine, methionine, valine, proline, alanine, hydroxyproline, threonine, glutamic acid, aspartic acid, glutamine, lysine, serine, asparagine, citrulline, and arginine). The amino acid content followed the order: peel (3.23%) > pulp (2.22%) > seeds (0.51%). Su *et al.* [53] measured the free amino acid content in different medicinal organs such as Trichosanthis Pericarpium and Trichosanthis Semen. The results showed that the amino acid content varied among different organs, with the pulp having a higher amino acid content than the seeds. Additionally, the *Trichosanthes* pulp is rich in polysaccharides, proteins, and other nutritional components, leading to the conclusion that it is a highly valuable raw material resource for medicinal purposes. Hu *et al.* [54] studied the nutritional components of Trichosanthis Semen, measuring the contents of 18 amino acids, including 8 essential amino acids for humans, which accounted for 29% of the total. They also determined the contents of 14 mineral elements, and the results showed high levels of trace elements required by the human body, such as calcium, iron, manganese, copper, and zinc, while harmful elements like arsenic and mercury were all below the national standard limits.

## Proteins and Peptides

Trichosanthis Fructus contains a large amount of protein, which has been extensively studied by scholars both at home and abroad. Casellas Pierre *et al.* [55] isolated and purified a ribosome-inactivating protein (RIP) from Trichosanthis Semen, with a molecular weight of approximately 27 kDa, named trichokirin (TCK). Five years later, Chinese scholar Wang *et al.* [56] also isolated and purified trichokirin. Falasca Anna Ida *et al.* [57] isolated a lectin from Trichosanthis Semen with a molecular weight of approximately 57. It is a glycoprotein composed of two subunits weighing 37 and 25 kDa, respectively. Uchikoba Tetsuya *et al.* [58] purified two serine proteases, named A and B, from *Trichosanthes* pulp, both with molecular weights around 50 kDa. Chinese scholar Dong[59] isolated a new RIP from Trichosanthis Semen with a molecular weight of 27.5 kDa, named β-kirilowin. Wong Ricky Ngok[60] isolated another new RIP from Trichosanthis Semen with a molecular weight of 28.8 kDa, named α-kirilowin. Tai Ningwen *et al.* [61] isolated and purified a novel small-molecule RIP from Trichosanthis Semen with a molecular weight of approximately 8 kDa, named S-trichokirin. Li *et al.* [62] isolated a new RIP from the seeds with a molecular weight of about 11 kDa, designated as trichokirin-S1. Shu *et al.* successfully purified another novel RIP from the seeds with a molecular weight of around 10.9 kDa, named trichosanthrip. Mi *et al.* [63] isolated a new RIP from Trichosanthis Fructus, named trichomislin.

## Other Components

Additionally, Trichosanthis Fructus contains compounds such as dibutyl malate (93)[28], 2, 5-dihydroxymethylfuran (94), 5-hydroxymethylfurfural (95), 5, 5-bis(oxymethyl)furfural (96), bluemenol A (97), cucumegastigmanes I (98)[37], and 2-methyl-3, 5-dihydroxy-tetrahydropyran-4-one (99)[36].

Trichosanthis Fructus contains a wide variety of chemical components with multiple pharmacological activities. Both domestic and international clinical and experimental data have confirmed that it has efficacy in treating coronary heart disease, angina pectoris, myocardial ischemia, and other conditions, and it also has a long history of medicinal use. However, the specific markers closely related to its therapeutic effects remain undetermined, and the lack of quality control indicator components has made it challenging to establish standardized quality criteria for *Trichosanthes* medicinal materials and related preparations. Therefore, we further explored the active fractions or components of Trichosanthis Fructus, Trichosanthis Pericarpium and Trichosanthis Semen, aiming to establish them as quality control markers. We also investigated new targets of bioactive compounds from Trichosanthis Fructus against myocardial ischemia, explored novel clinical applications, and ultimately pursued new drug development.

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## Harvest

### Harvesting time

The harvest period occurs when soybean leaves turn yellow and fall off, stems become dry, pods turn brown, and seeds harden to exhibit their characteristic color and shape[14]. At this stage, the grain moisture content typically ranges between 18% and 20%. Premature harvesting results in immature seeds with high moisture content and reduced 100-seed weight, negatively impacting yield and quality. Delayed harvesting increases the risk of pod shattering, leading to yield losses.

### Harvesting methods

Currently, soybean harvesting primarily involves two methods: mechanical harvesting and manual harvesting. Mechanical harvesting is highly efficient and suitable for large-scale planting areas[15]. When using a combine harvester, it is essential to adjust the machine's parameters to ensure harvesting quality and minimize losses. Manual harvesting is applicable for small-scale planting areas or fields where machinery cannot operate. During manual harvesting, care should be taken to cut and handle the plants gently to avoid soybean loss. After harvesting, the soybeans should be promptly dried to reduce moisture content to below 13%, followed by threshing, cleaning, and storage.

## Conclusions

The high-quality and high-yield cultivation techniques for summer soybean are a systematic project, involving multiple aspects such as variety selection, seed treatment, field selection and land preparation, sowing techniques, field management, pest and disease control, and harvesting. Only by strictly following scientific methods in every step can the yield potential of summer soybeans be fully realized, achieving the goal of high quality and high yield. In actual production, farmers should flexibly apply cultivation techniques based on local conditions, continuously summarize experience, and improve their planting level to contribute to the development of China's soybean industry.

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