Research Progress of Triterpenoid Secondary Metabolites in Cucurbitaceae Plants

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Abstract In this paper, the advances in study on triterpenoids in *Trichosanthes*, *Hemsleya*, *Gynostemma*, *Actinostemma* and *Siraitia* Merr. plants were reviewed. Terpenoids are the main secondary metabolites of Cucurbitaceae and have obvious pharmacological activity. It is noteworthy that in these plants, there are a variety of triterpenoids, which are diverse in structure. Triterpenoid saponins have greater potential for development.

Key words Cucurbitacea; Triterpenoids; Secondary metabolites; *Trichosanthes*; *Hemsleya*; *Gynostemma*; *Actinostemma*; *Siraitia* Merr.

Cucurbitaceae is one of the important edible plant families, and its importance is second only to Gramineae, Leguminosae and Solanaceae. There are 154 species and 35 varieties of 32 genera in China, and there are about 29 species recorded in literatures[1] as local medicines and folk medicines, including commonly used Chinese herbal medicines such as *Siraitia grosvenorii*, *Trichosanthes kirilowii* Maxim. and *Bolbostemma paniculatum* (Maxim.) Franquet[2]. The main secondary metabolites of Cucurbitaceae are triterpenoids with significant physiological activity. The triterpenoid structures that have been found mainly include cucurbitane type tetracyclic triterpenoid saponins such as mogroside V, dammarane type saponins such as *Actinostemmoside* A and gypenoside I, and pentacyclic triterpenoid oleanane type saponins. In recent years, many new triterpenoids have been discovered in Cucurbitaceae. In this paper, the triterpenoid structures of several important genera in Cucurbitaceae were summarized, so as to provide a chemical basis for further studying the structure-activity relationship of cucurbitaceous active components and finding new drug sources in closely related plants and to lay a foundation for the full development and utilization of cucurbitaceous drug resources.

In this paper, we summarized the triterpenoid secondary metabolites in *Trichosanthes*, *Hemsleya*, *Gynostemma*, *Actinostemma* and *Siraitia* in Cucurbitaceae. The triterpenoid aglycones found in the five genera were mainly cucurbitane, dammarane, cycloartane and oleanane. The abbreviations shown in the represent paper are as follows: Gyp: gypenoside, glc: β-D-glucose, ara: α-L-arabinose, rha: α-L-rhamnose, xyl: β-D-xylose, Ac :acetyl).

Trichosanthes

The *Trichosanthes* has a long medicinal history. The traditional Chinese medicines, Cotex Trichosanthis, Semen Trichosanthis, Fructus Trichosanthis and Radix Trichosanthis recorded in *Chinese Pharmacopoeia*, are dried peels, seeds, fruit and roots of *T. kirilowii* in Cucurbitaceae, respectively[2]. There are more than 50 kinds of terpenoids, mainly belonging to tetracyclic triterpenoids (cucurbitane and cycloartane types), and pentacyclic triterpenoids (oleanane type). Two new cycloartane structures were found from the seeds of *T. kirilowii*, *i. e.*, dihydroxycycloalkane-type triterpenoids with one hydroxyl group and one tetrahydrofuran ring on the two C-17 side chains.

Tetracyclic triterpenoids

The tetracyclic triterpenoids of the genus *Trichosanthes* have cucurbitane type and cyclopentane type structures. According to the nuclear structures of the cucurbitane type, they can be subdivided into Class I and Class II. Class I: C-1(2) and C-5(6) form two endo-double bonds, and the structure of the mother nucleus is shown in Fig. 1. The structural characteristics of these compounds are that the C-2 position is substituted by a hydroxyl group or a glycosyl group, and the C-16 position is substituted by a carbonyl group or a hydroxyl group or the side chain at the C-17 position forms a ring, for instance, the compounds khekadaengoside G and tricuspidatin[3-4] represented by the structures T-1 and T-3 have the side chain formed an oxygen-containing six-membered ring or the side chain formed a ring with the hydroxyl group at the C-16 position. Class II: C-5(6) forms an endo-double bond. The structure of the mother nucleus is shown in Fig. 1. The characteristics of these compounds are that the C-17 position is substituted by different side chains, and the C-2, C-3 and C-16 positions are substituted by different substituents. The cyclopentane type tetracyclic triterpenoids found in the genus *Trichosanthes* include cyclotricuspidosides B, cyclokirilodiol and isocyclokirilodiol[5-6], and the latter two compounds are new structures of the cyclodecane type, as shown by T-4 and T-5.

Pentacyclic triterpenoid

The genus *Trichosanthes* mainly contains oleanane type pentacyclic triterpenoids such as 7-oxodihydrokarounidiol, karounidios-3-O-benzoate and 3, 29-O-dibenzoyloxykarounidiol[7-12], which

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Zhiyi HU (1992 -), female, master, devoted to research about extraction and activity of chemical components from traditional Chinese medicine.

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are mainly found in the seeds of *T. kirilowii*, and there are also compounds obtained after processing. According to the number and position of double bonds in the structures, they can be divided into different types. From the perspective of the mother nucleus structures, isokarounidiol and karounidiol may be formed from 6-hydroxydihydrokarounidiol through dehydration and migration of the double bond following dehydration, respectively. In addition, a rare $\Delta^{5,7,9(11)}$-conjugated triene system naturally occurring in the triterpenoid compounds, *i. e.*, 5-dehydrokarounidiol, was found in *T. kirilowii*.

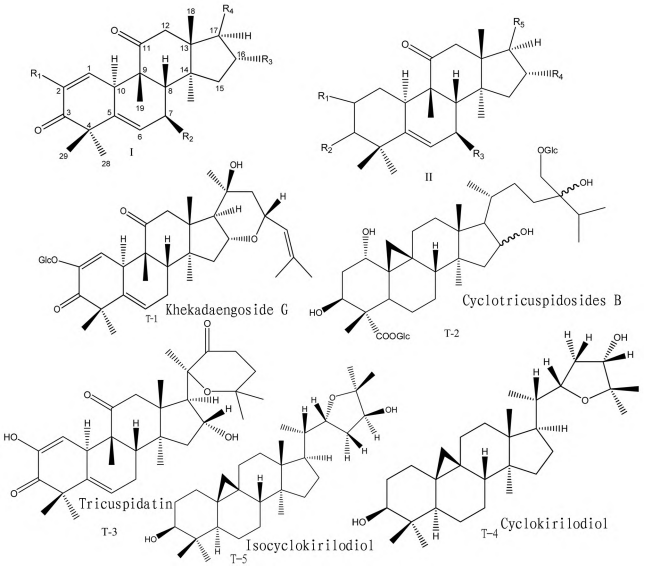

Fig. 1 Structures of tetracyclic triterpenoids in *Trichosanthes*

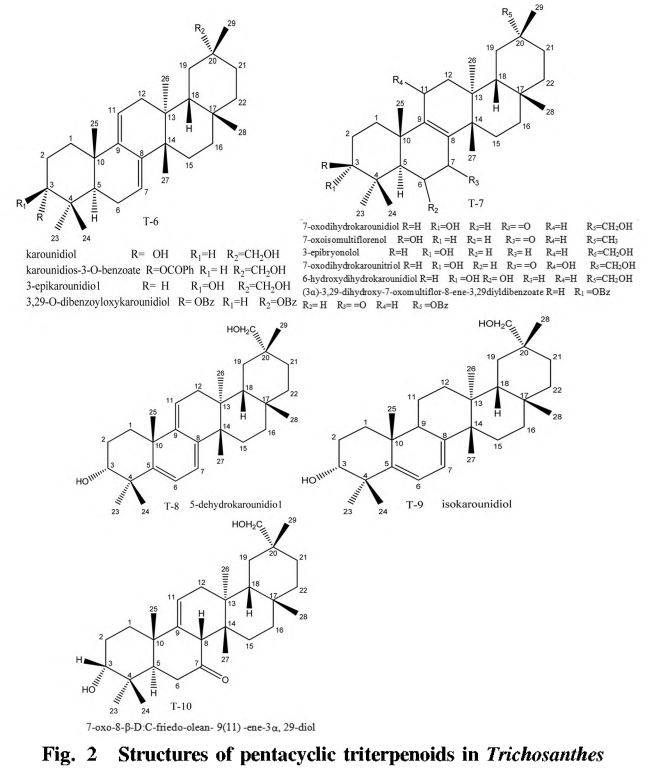

Fig. 2 Structures of pentacyclic triterpenoids in *Trichosanthes*

Hemsleya

There are about 30 species in *Hemsleya*, and this genus has a variety of tubers for medicinal purposes, which are used as raw materials for extracting *hemsleya*din or as a raw medicinal material[1]. *Hemsleya* is rich in triterpenoids, mainly cucurbitane type tetracyclic triterpenoids and their glycosides and oleanane type pentacyclic triterpenoids and their glycosides. At present, researchers at home and abroad have isolated more than 80 cucurbitane type triterpenoids from this genus. Glycosides are often connected to 1 – 3 glucoses, the collecting positions of which are mostly at C-2 and C-3 positions and the 26 and 27 positions of the side chain, and partial structures are shown in Fig. 3 and Table 1. The nucleus of this genus is mainly tetracyclic triterpenoid, and the structural features are that the C-2 and C-3 positions are glycosidated or substituted by hydroxy, the C-11 position has hydroxyl or carbonyl, the C-23 and 24 positions of the side chain often have double bonds, and the 25 position has hydroxyl or acetyl group. The other major class of triterpenoids in *Hemsleya* is oleanane type compounds, about 30 kinds, and the nucleus structure is shown as T-18. The differences between these compounds are mainly that the substituents at the C-3 and C-28 positions are different, the 3 position contains a glycosyl group , and the 28 position is mainly connected via a carboxyl group on the aglycone to glucose or glucuronic acid, arabinose and mannose rarely, such as 3-O-(6′-butyl ester-)-β-D-glu-curonopyranosyl)-oleanolic acid-28-O-α-L-arabinopyranoside, Hemslonin B and 3-O-β-D-glucuropyranosyl oleanolic acid-28-O-β-Dmanupyranoside[16, 29].

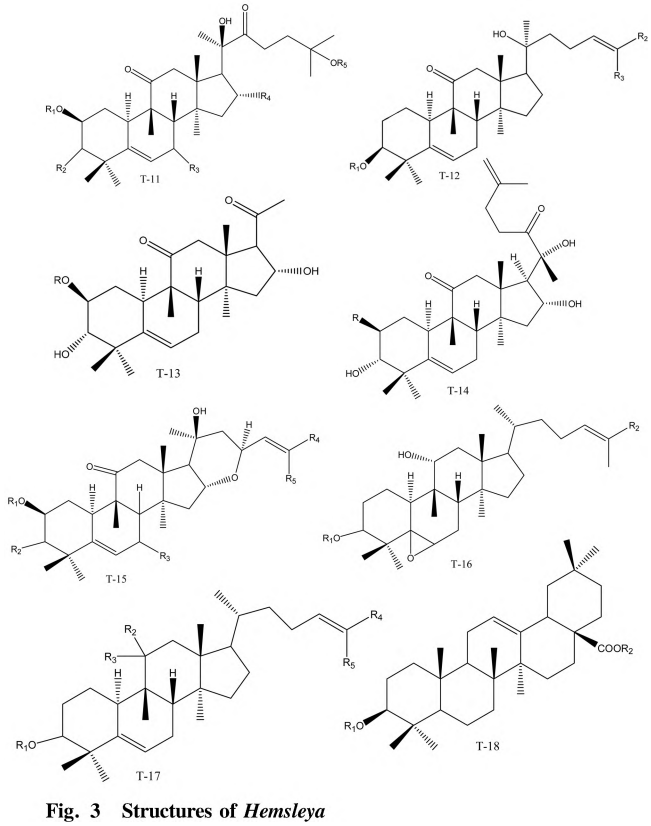

Fig. 3 Structures of *Hemsleya*

Table 1 Triterpenoids of Hemsleya

Number	Compound name	Nuclear structure	r_1	r_2	r_3	r_4	r_5	Reference
1	Hemslecin A	t-11	h	α-oh	h	oh	ac	[13]
2	Hemslecin B	t-11	h	α-oh	h	oh	h	[13]
3	2-O-β-D-glycoside of hemslecin A	t-11	glc	α-oh	h	oh	ac	[14]
4	Hemsamabilinin B	t-11	glc	α-oh	h	oh	h	[15]
5	Hemslecins g	t-11	h	α-oh	oh	oh	ac	[16]
6	23,24-dihydro cucurbitacin f-16,25-diacetate	t-11	h	oh	h	$ococh_3$	ac	[17]
7	23,24-dihydro cucurbitacin f-16,25-diacetate-2-o-α-d-glucopyranoside	t-11	glc	oh	h	$ococh_3$	ac	[17]
8	23,24-dihydro cucurbitacin f-16-acetate	t-11	h	oh	h	$ococh_3$	h	[17]
9	Scandenoside r_1	t-12	glc	ch_2oh	ch_3			[18]
10	Scandenoside r_2	t-12	glc	ch_3	ch_2oh			[18]
11	Scandenoside r_3	t-12	glc	ch_2o-glc	ch_3			[19]
12	Scandenoside r_4	t-12	glc	ch_3	ch_2o-glc			[19]
13	Delavanosideb	t-12	h	ch_3	ch_2o-glc_2-glc			[20]
14	Delavanosidec	t-12	glc	ch_3	ch_2o-glc_2-glc			[20]
15	Scandenoside r_9	t-13	glc					[23]
16	Xuedanglycoside b	t-14	glc					[24]
17	Scandenoside r_{10}	t-15	h	glc	h	ch_2oh	ch_2oh	[25]
18	Xuedanglycoside a	t-15	glc	α-oh	h	ch_3	ch_3	[26]
19	Scandenoside r_{11}	t-16	glc	ch_2o-glc_6-glc				[25]
20	Scandenoside r_5	t-17	glc		o	ch_3	ch_2o-glc_2-glc	[27]
21	Scandenoside r_6	t-17	glc	h	α-oh	ch_3	ch_2o-glc_2-glc	[19]
22	Scandenoside r_7	t-17	glc	h	α-oh	ch_3	ch_2o-glc_6-glc	[27]
23	Scandenoside r_8	t-17	glc		o	ch_2o-glc	ch_3	[19]
24	Delavanosidea	t-17	h		o	ch_3	ch_2o-glc_2-glc	[20]
25	Delavanosided	t-17	glc		o	ch_2oh	ch_3	[20]
26	Delavanosidee	t-17	glc		o	ch_2o-glc_2-glc_6- glc	ch_3	[20]
27	Carnosifloside vi	t-17	glc	h	α-oh	ch_2o-glc_6-glc	ch_3	[21]
28	Jinfushanoside a	t-17	glc	h	α-oh	ch_2oh	ch_2oh	[22]
29	Jinfushanosideb	t-17	glc		o	ch_2o-glc	ch_2oh	[22]
30	Jinfushanosidec	t-17	glc		o	ch_2o-glc	ch_2oh	[22]
31	Jinfushanosided	t-17	h		o	ch_2oh	ch_2oh	[22]
32	Oleanolic acid -28-o-β-d-glucopyranoside	t-18	h	glc				[28]

Gynostemma

According to *Flora of China*, there are 11 species and 2 varieties of *Gynostemma* in China[1]. *Gynostemma* triterpenoids have the same basic skeleton as ginsenosides (dammarane type). The secondary metabolites of *Gynostemma* triterpenoids are mainly saponins, in addition to flavonoids, polysaccharides and sterols. *G. pentaphyllum* contains a variety of saponin components, and more than 140 kinds are currently isolated. There are 83 dammarane-type tetracyclic triterpenoid structures similar to ginsenosides, and among them, gypenosides III, IV, VIII and XII are exactly the same as ginsenosides Rb1, Rb3, Rd and F2.

The dammarene double bond of gypenosides is mostly at the 24 and 25 positions of the side chain. The gypenosides are mostly glycosidated at the C-3 and C-20 positions, with some of the side chains forming a ring. There are several types of ring formation. The first one: the five-membered ring of lactone structure is shown as T-19; the second one: an epoxy five-membered ring structure is formed at the 20 and 24 positions, as shown by structure T-20; the third one: the 20 and 25 positions have an epoxy six-membered ring, as shown by structure T-21; the fourth one: the 21 and 24 positions form a five-membered ring, which has two hydroxyl groups thereon, as shown by structure T-22; the fifth one: the 21 and 23 positions form an oxygen-containing five-membered ring, as shown by structure T-23; the sixth one: the 21 and 24 positions of the side chain form a five-membered ring, which has three hydroxyl groups thereon, as shown by structure T-24; and the seventh one: the 24 position is simultaneously conjugated to the 12 and 20 positions, forming oxygen-containing rings, as shown by structure T-25. The compounds are given in Table 2.

Actinostemma

The seeds and whole herb of *Actinostemma tenerum* Griff. are medicinal, and can be used as a raw material containing dammarane type saponins similar to ginsenosides in addition to Araliaceae, like *G. pentaphyllum*[1]. So far, more than 30 triterpenoids have been isolated from the *A. tenerum*, mainly dammarane, baccharane, and oleanane[33-40]. Among them, a cucurbitane type structure, cucurbitacin E, was also found in the genus. There are 8 kinds of dammarane compounds found in *Actinostemma*, of which

actinostemmoside I and actinostemmoside J are new compounds, and actinostemmoside I and actinostemmoside C are isomers, which are different in double bond configuration. The structural differences between actinostemmoside J and others are that the C-3 position is collected to a glycosyl group and the C-20 position is collected to a hydroxyl group. The oleanane triterpenoids in *Actinostemma* are in the form of glycosides, mainly tetraglycosides and pentaglucosides, and very few are hexasaccharides and heptaglucosides. And the glycosyl groups are glucose, rhamnose, arabinose, galactose and xylose.

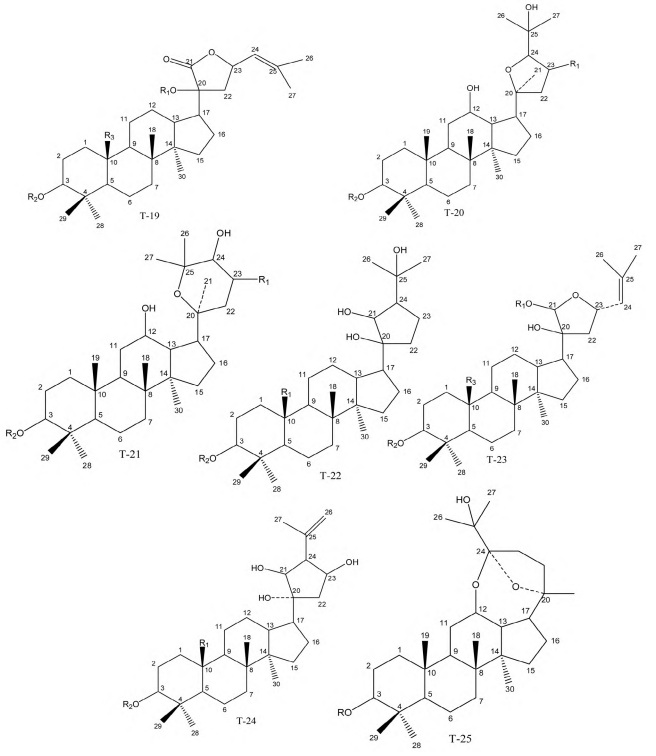

Fig. 4 The nuclear structures of triterpenoids in *Gynostemma*

Siraitia Merr

There are four species of *Siraitia* Merr. in China, of which only *S. grosvenorii* and *S. siamensis* are used as a medicine, while more reports are focused on *S. grosvenorii*. *S. grosvenorii* is a geo-authentic crude drug in Guangxi Province, as well as a natural sweetener with sweetness and low calorie. Its sweet components are mainly cucurbitane type tetracyclic triterpenoids, and the glycosyl connected to the aglycones is glucose. The differences in glycoside compounds are the connecting position of glucose and the number of glycosyl groups in the structure. The fruit is rich in triterpenoid saponins, and the mogroside IV, mogroside V, and siemanoside I are much sweeter than sucrose. In addition, oleanane type triterpenoid benzoate was also found, as shown by T-38 and T-39.

The tetracyclic triterpenoid saponins isolated from *S. grosvenorii* have sweet taste (such as mogroside V), bitter taste (such as mogroside II), or are tasteless (such as mogroside III). Their differences lie in the number, position and type of glycosyl groups. In addition, a series of triterpenic acids have been isolated from the root of *S. grosvenorii*, and the structures are new, as shown in

Table 2 Triterpenoids of *Gynostemma*

Number	Compound name	Stucture	R_1	R_2	R_3	Reference
1	Compd. 4	T-19	H(R)	glc^2-rham | 3 xyl		[29]
2	Compd. 5	T-19	H(S)	glc^2-rham | 3 glc $COCH_3$ 3 |		[29]
3	Compd. 6	T-19	H(R)	glc^2-rham 6 | | 4 Xyl $COCH_3$		[29]
4	Gynoside A	T-20	H(S)	glc^6-xyl		[30]
5	Gynoside B	T-20	H	glc^6-glc		[30]
6	Gynoside C	T-20	H(R)	glc^2-xyl		[30]
7	Gynoside D	T-20	OH(R)	glc^2-xyl		[30]
8	Compd. 10	T-21	OH	glc^2-glc | 6 xyl		[31]
9	Compd. 11	T-21	OH	Xyl^2-glc		[31]
10	Compd. 13	T-21	OAc	xyl^2-xyl		[31]
11	Compd. 14	T-21	OAc	glc^2-xyl		[31]
12	Compd. 15	T-21	OAc	glc^2-xyl | 6 xyl		[31]
13	21, 24-cyclopentyldammarane of 8	T-22	CH_3	$COCH_3$ 6 | glc^2-rham | 3 xyl		[32]
14	21, 24-cyclopentyldammarane of 9	T-22	CHO	ara^2-rham | 3 xyl		[32]
15	Compd. 1	T-23	H	ara^2-rham | 3 xyl	CHO	[32]
16	Compd. 2	T-23	H	glc^2-rham | 3 xyl	CH_3	[32]
17	Compd. 4	T-23	Et	ara2-rham | 3 xyl	CHO	[32]
18	Compd. 5	T-23	Et	glc^2-rham | 3 xyl	CH_3	[32]
19	Aglycons 21,24-cyclopentyldammar-25-ene of 6	T-24	CH_3	OAc 6 | glc^2-rham | 3 xyl		[32]
20	Aglycons 21,24-cyclopentyldammar-25-ene of 7	T-24	CHO	ara^2-rham | 3 xyl		[32]
21	Gynoside E	T-25	glc2-xyl			[30]

T-36 of Fig. 6. Up to now, more than 30 triterpenoids have been isolated from *Siraitia* plants[41, 60].

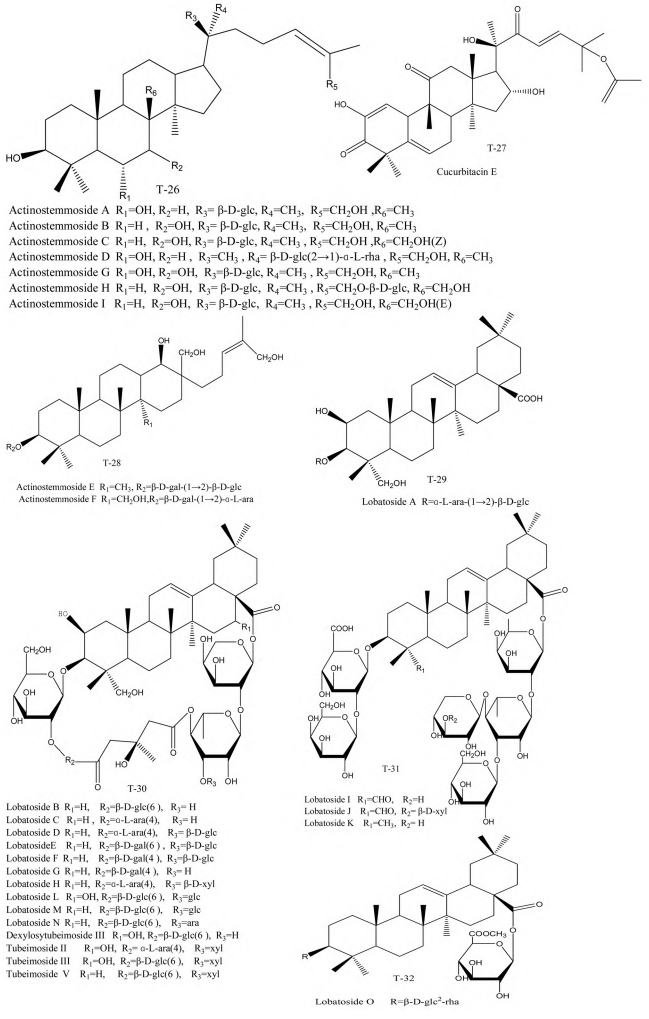

Fig. 5 The structure of triterpenoids in *Actinostemma*

Conclusions

In this paper, the basic structural characteristics of triterpenoids in *Trichosanthes*, *Hemsleya*, *Gynostemma*, *Actinostemma* and *Siraitia* Merr. in Cucurbitaceae were summarized, and the secondary metabolites of the triterpenoids have obvious differences between species. Among them, triterpenoids in *G. pentaphyllum* and *A. tenerum* were found to have a structure similar to that of ginsenosides, and triterpenoids in *S. grosvenorii* was found to have a sweetness far exceeding that of sucrose due to the particularity of the cucurbitane type structure. On the other hand, the secondary metabolites of triterpenoids in different genera have certain commonality in structure, which provides a chemical basis for kinship. In view of the particularity of triterpenoid structures in Cucurbitaceae plants, it is necessary to further study Cucurbitaceae plants in the future. *S. grosvenorii* is an important natural product in Cucurbitaceae regarded as both medicine and food. However, few studies have been conducted on its roots, and we can turn the target to the roots of *S. grosvenorii* and to study its medicinal components.

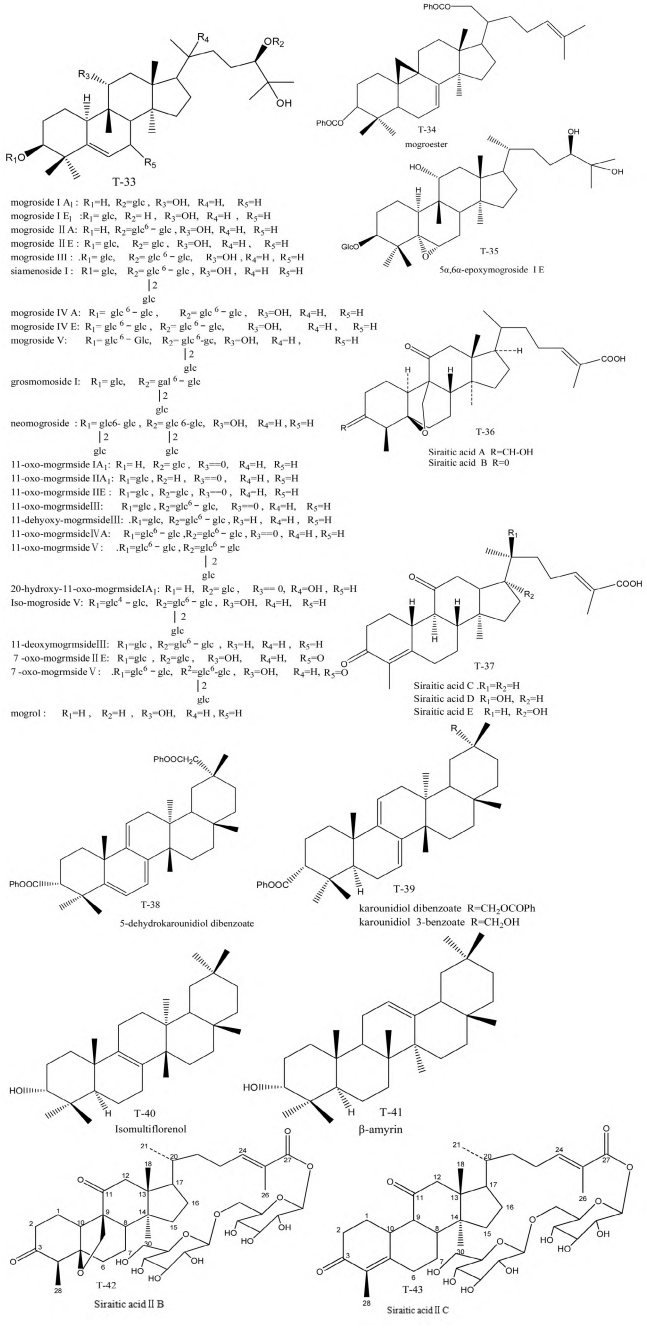

Fig. 6 The structure of triterpenoids in *Siraitia* Merr.

The difference of secondary metabolites is the main reason for the difference in activity, and it is also the benchmark for determining clinical application. Through the summary of the secondary metabolite systems in different genera of Cucurbitaceae, we can deeply study the structure-activity relationship on this basis, and conduct in-depth development and utilization of Cucurbitaceae plant resources.

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It could be seen from Table 6 that combinations 03786 × Wva536 and 05237 × Wva536 had a higher negative value, and were better combinations in tomato breeding for resistance to tomato gray mold. Therefore, in the tomato breeding for resistance to tomato gray mold, the selection of general combining ability should be emphasized, and then the selection of special combining ability should be considered.

Estimation of genetic parameters

The estimated genetic parameters of complete diallel cross are shown in Table 7.

Table 7 Estimation of genetic parameters

Trait	Genetic variance	Additive variance	Dominant variance	Environmental variance	Phenotypic variance	Broad-sense heritability	Narrow-sense heritability
Disease index	396.118 6	359.987 3	33.237 6	5.513 2	405.336 7	89.73%	82.15%

It could be seen from Table 7 that the additive variance was much larger than the dominant variance, indicating that the additive effect of resistance genes played a major role, and the environmental variance was larger, indicating that the expression of resistance was greatly affected by the environment. The broad-sense heritability and narrow-sense heritability were, respectively, 89.73% and 82.15%, which were both higher, indicating that the parents had a greater effect on offsprings. When formulating hybrid combinations, the traits of the parents have a greater impact on offsprings. In the early generations of hybrids, the effect is more obvious, and the space for genetic breeding using resistance genes is larger.

Discussion

There are significant differences in the genetic basis between the test materials. The F_1 generation of resistant and susceptible parents or susceptible and resistant parents showed intermediate or slightly higher resistance, and the disease resistance was incompletely dominant with high heritability, which is consistent with previous studies. The general combining ability and special combining ability of the tested varieties reached the extremely significant level. The general combining ability variance was significantly larger than the special combining ability variance, GCA/SCA = 26.9 > 4, indicating that tomato resistance to gray mold was genetically additive, the dominant effect was in a secondary position, and there was a cytoplasmic effect. Therefore, tomato breeding for resistant to gray mold should mainly depend on cross breeding.

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