

Review

Systematic Review of Chemical Constituents in the Genus *Lycium* (Solanaceae)

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Abstract: The *Lycium* genus is widely used as a traditional Chinese medicine and functional food. Many of the chemical constituents of the genus *Lycium* were reported previously. In this review, in addition to the polysaccharides, we have enumerated 355 chemical constituents and nutrients, including 22 glycerogalactolipids, 29 phenylpropanoids, 10 coumarins, 13 lignans, 32 flavonoids, 37 amides, 72 alkaloids, four anthraquinones, 32 organic acids, 39 terpenoids, 57 sterols, steroids, and their derivatives, five peptides and three other constituents. This comprehensive study could lay the foundation for further research on the *Lycium* genus.

Keywords: *Lycium* genus; chemical constituents; goji berry; Lycii cortex

1. Introduction

Lycium is one of the genera in the Solanaceae family, comprising 80 species, seven of which are found in China [1]. These species are all deciduous shrubby, possessing a highly similar morphology and structure. The *Lycium* genus has been an important source of medicines and nutrient supplements for thousands of years in Southeast Asia, especially in China. Two species in particular, *Lycium barbarum* and *Lycium chinense*, have been widely used as traditional Chinese medicinal herbs for centuries and *L. barbarum* is currently widely cultivated in China.

Goji berries (Chinese name Gouqizi), which are derived from the fruits of *Lycium* Linn, have been used as traditional herbs for a long time in China for their benefits of replenishing vital essence to improve eyesight, nourish the liver and kidneys. Lycii cortex is a “heat cleansing” drug that is derived from the root bark of *L. chinense* and *L. barbarum* [2]. Goji berries and Cortex Lycii have demonstrated good therapeutic effects in some chronic diseases such as hectic fever, night sweats, cough, hemoptysis, and diabetes. Recently, medical research has indicated that these fruits and root bark have many pharmacological functions, such as antiglaucoma, immunoregulatory, antitumor, antioxidant, antiaging, neuroprotective, and blood sugar level reducing activities [3–10].

Traditionally, the berry and root bark available have been used as medicinal sources, as well as important components in some traditional Chinese patent medicines. They are not only famous medical herbs, but are also functional foods widely consumed in health-preserving cuisines, i.e., soups, congee, herbal tea, etc. People also eat the fresh leaves as vegetables. In particular, goji berries have become increasingly popular for improving overall well-being and as an anti-aging remedy. There are many goji derived-products on health food market, such as dried fruits, juice, goji wine and goji yoghurt. Many research papers were published focused on the phytochemical fingerprinting and antioxidant activity of these products [11–14].

Two valuable medicinal herbs, namely *L. barbarum* and *L. chinense*, have received remarkable attention due to their effective clinical therapy, especially in the anti-aging category. In addition, there are increasing numbers of publications about several other *Lycium* plants, i.e., *Lycium ruthenicum* [15,16]. Many researchers have focused great attention on the *Lycium* genus in recent years, and many chemical components from this genus have been isolated. Therefore, a comprehensive and systematic review on the chemical constituents of the *Lycium* genus is much needed.

Most of the published reviews not only covered chemical composition, but also summarized the pharmacology, clinical studies, safety, toxicology and adverse actions of *L. barbarum* or *L. chinense* [17–19]. The aim of this review was to focus on chemical constituents in different parts of plants from different species in *Lycium* genus, especially small molecular compounds with updated research reports. This paper comprehensively summarizes the reports of constituents from the genus *Lycium*. Up to 2016, at least 355 constituents were reported from different species in the *Lycium* genus and different parts (fruits, root bark, leaves, seeds, and flowers) of the plant. This review describes the advances in the phytochemistry of the genus *Lycium* from 1975 to 2016, based on the 142 cited references. The reported constituents can be classified as glycerogallactolipids, phenylpropanoids, coumarins, lignans, flavonoids, amides, alkaloids, anthraquinones, organic acids, terpenoids, sterols, steroids, peptides, and other constituents. The aim of this review is to illustrate the recent advances in the characterization of the *Lycium* genus. The results, based on these phytochemical studies, could lay a solid foundation for better understanding of pharmacological activities of *Lycium* and quality assessment.

2. Constituents

Until now, other than polysaccharides, more than 355 compounds have been isolated and identified from the *Lycium* genus. The small molecules can be assigned to various classes of glycerogallactolipids, phenylpropanoids, coumarins, lignans, flavonoids, amides, alkaloids, anthraquinones, organic acids, terpenoids, sterols, steroids and their derivatives, and peptides. Beyond that, other groups of compounds have also been reported. The proportion of different compounds of the *Lycium* genus is shown in Figure 1. Their structures are shown below, and their names and corresponding plant sources are included in this paper.

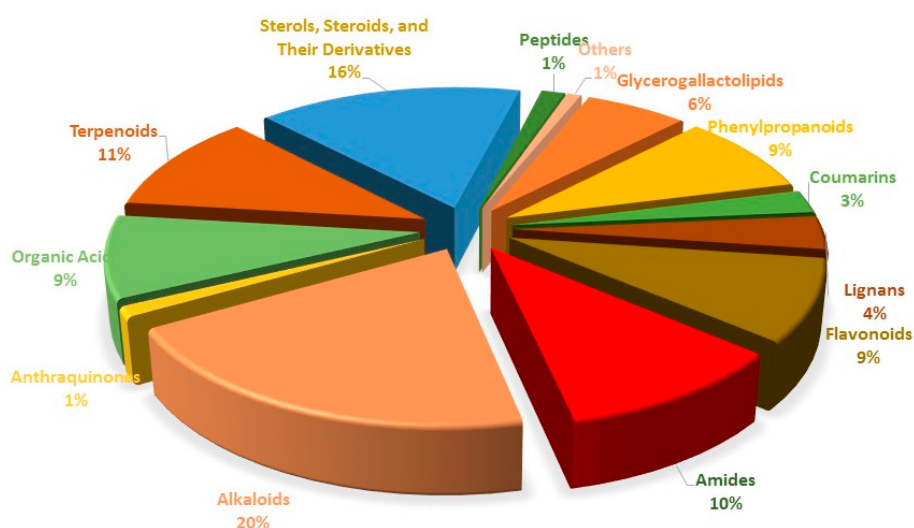


Figure 1. Different subtype comparison of the 355 constituents reported from *Lycium* genus.

2.1. Macromolecules in the *Lycium* Genus

Polysaccharides

Polysaccharides are the most important group of substances in the goji berry, which are estimated to comprise 5–8% of the dried fruits [20], 1.02–2.48% of the raw material [21–23]. More than 40 polysaccharides, with a molecular weight range of 8–241 kDa, were isolated from the fruit of *L. barbarum*, *L. chinense* and *L. ruthenicum*. Two, LRLP4-A and LBLP5-A, were isolated from the leaves of *L. ruthenicum*. The polysaccharides share a glycan-O-Ser glycopeptide structure and contain galacturonic acid, 18 amino acids, and nine monosaccharides, namely, xylose (Xyl), glucose (Glc), arabinose (Ara), rhamnose (Rha), mannose (Man), galactose (Gal), fucose (Fuc), galacturonic acid (GalA), glucuronic acid (GlcA) [24]. The molar ratios of the polysaccharides are shown in Table 1. The polysaccharides can be isolated and purified by water extract alcohol precipitation, DEAE ion-exchange cellulose, gel-permeation chromatography, high performance liquid chromatography (HPLC). Seavage method and organic reagents were used to remove proteins, pigments and other impurities. The structural composition of a LBP can be studied by SDS-PAGE gel electrophoresis, high performance size exclusion chromatography (HPSEC), gas-chromatographic-mass-spectrometry (GC-MS), nucleic magnetic resonance (NMR), and matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-Tof-MS) [18,21,25].

Table 1. The molar ratios and source of LBPs.

LBPs	Molar Ratio	Source	Reference
LbGp1	Ara:Gal:Glc = 2.5:1.0:1.0	<i>L. barbarum</i>	[26]
LbGp2	Ara:Gal = 4:5	<i>L. barbarum</i>	[27]
LbGp3	Ara:Gal = 1:1	<i>L. barbarum</i>	[28,29]
LbGp4	Ara:Gal:Rha:Glc = 1.5:2.5:0.43:0.23	<i>L. barbarum</i>	[28,30]
LbGp5	Rha:Ara:Xyl:Gal:Man:Glc = 0.33:0.52:0.42:0.94:0.85:1	<i>L. barbarum</i>	[28]
LbGp5B	Rha:Ara:Glc:Gal = 0.1:1:1.2:0.3	<i>L. barbarum</i>	[31]
LBP3p	Rha:Ara:Xyl:Gal:Man:Glc = 1.25:1.10:1.76:1:1.95:2.12	<i>L. barbarum</i>	[32]
LBPC ₂	Xyl:Rha:Man = 8.8:2.3:1	<i>L. barbarum</i>	[33]
LBPC ₄	Glc	<i>L. barbarum</i>	[33]
LBPA1	heteroglycan	<i>L. barbarum</i>	[33]
LBPA3	heteroglycan	<i>L. barbarum</i>	[33]
LBP1a-1	Glc	<i>L. barbarum</i>	[34]
LBP1a-2	Glc	<i>L. barbarum</i>	[34]
LBP3a-1	GalA	<i>L. barbarum</i>	[34]
LBP3a-2	GalA	<i>L. barbarum</i>	[34]
LBPF1	-	<i>L. barbarum</i>	[35]
LBPF2	-	<i>L. barbarum</i>	[35]
LBPF3	-	<i>L. barbarum</i>	[35]
LBPF4	-	<i>L. barbarum</i>	[35]
LBPF5	Ara, Man, Xyl, Glu, Rha	<i>L. barbarum</i>	[35,36]
LBPF6	-	<i>L. barbarum</i>	[36]
LPBC4	Glc	<i>L. barbarum</i>	[37]
LBP-1	Rha:Ara:Xyl:Gal:Man:GalA = 1:7.85:0.37:0.65:3.01:8.16	<i>L. barbarum</i>	[22]
WSP1	Rha:Fuc:Ara:Xyl:Man:Gal:Glc = 1.6:0.2:51.4:4.8:1.2:25.9:7.3	<i>L. barbarum</i>	[23]
AGP	Rha:Ara:Xyl:Gal:Glc:GalA:GlcA = 3.3:42.9:0.3:44.3:2.4:7.0	<i>L. barbarum</i>	[38]
LBP-IV	Rha:Ara:Xyl:Glc:Gal = 1.61:3.82:3.44:7.54:1.00	<i>L. barbarum</i>	[39]
LbGp1	Ara:Gal = 5.6:1	<i>L. barbarum</i>	[40]
LBP-s-1	Rha:Ara:Xyl:Man:Glu:Gal:Gal A = 1.00:8.34:1.25:1.26:1.91:7.05:15.28	<i>L. barbarum</i>	[41]
p-LBP	Fuc:Rha:Ara:Gal:Glc:Xyl:Gal A:Glc A = 1.00:6.44:54.84:22.98:4.05:2.95:136.98:3.35	<i>L. barbarum</i>	[42]
Cp-2-A	Ara:Gal:Man:Rha:Glu = 6.02:2.71:1.00:0.70:0.67	<i>L. chinense</i>	[43,44]
Cp-2-B	Ara:Gal = 1:0.96	<i>L. chinense</i>	[43,44]
Hp-2-A	Ara:Gal = 5.2:1	<i>L. chinense</i>	[43,44]

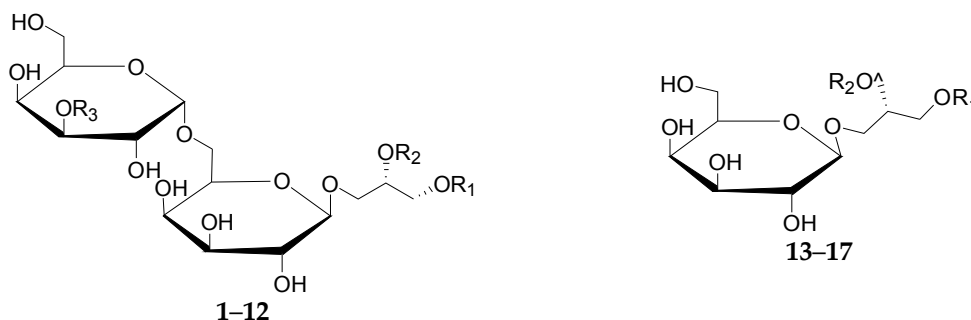
Hp-2-B	Ara:Gal = 7.9:1	<i>L. chinense</i>	[43,44]
Hp-2-C	Ara:Gal = 1.2:1	<i>L. chinense</i>	[43,44]
Hp-0-A	Ara:Gal = 14:1	<i>L. chinense</i>	[43,44]
Cp-1-A	Ara:Xyl = 1:1	<i>L. chinense</i>	[45]
Cp-1-B	Ara	<i>L. chinense</i>	[45]
Cp-1-C	Ara:Gal = 3:1	<i>L. chinense</i>	[45]
Cp-1-D	Ara:Gal = 1:1	<i>L. chinense</i>	[45]
LRGP1	Rha:Ara:Xyl:Man:Glu:Gal = 0.65:10.71:0.33:0.67:1:10.41	<i>L. ruthenicum</i>	[46]
LRGP2	-	<i>L. ruthenicum</i>	[47]
LRGP3	Rha:Ara:Gal = 1.0:14.9:10.4	<i>L. ruthenicum</i>	[48]
LRGP4-A	Rha:Ara:Glu:Gal = 1:7.6:0.5:8.6	<i>L. ruthenicum</i>	[49]
LRGP5	Rha:Ara:Xyl:Gal:GalA = 1.0:2.2:0.5:1.2:4.7	<i>L. ruthenicum</i>	[50]
LRLP4-A	Rha:Ara:Gal = 1:10.3:5.3	<i>L. ruthenicum</i>	[47]
LBLP5-A	-	<i>L. ruthenicum</i>	[51]

2.2. Small Molecule Substances

2.2.1. Glycerogalactolipids 1–22

At present, 17 compounds of this type, a series of glycerogalactolipids **1–17**, listed in Table 2, have been isolated and identified. Compounds **1–15** have been isolated and identified from the fruits of *L. barbarum* [52], whereas **16** and **17** were isolated from the fruits of *L. chinense* [53]. Compounds **18–22**, illustrated in Figure 2, were isolated from the root bark of *L. chinense* [54,55].

Table 2. Chemical structures of compounds **1–17**.



No.	Compounds	R ₁	R ₂	R ₃	Source
1	Glycerogalactolipids A	Palmitoyl	Linolenoyl	Linolenoyl	<i>L. barbarum</i>
2	Glycerogalactolipids B	Palmitoyl	Linolenoyl	Linoleoyl	<i>L. barbarum</i>
3	Glycerogalactolipids C	Palmitoyl	Linolenoyl	Palmitoyl	<i>L. barbarum</i>
4	Glycerogalactolipids D	Palmitoyl	Linoleoyl	Palmitoyl	<i>L. barbarum</i>
5	Glycerogalactolipids E	Palmitoyl	Palmitoyl	Palmitoyl	<i>L. barbarum</i>
6	Glycerogalactolipids F	Palmitoyl	Palmitoyl	H	<i>L. barbarum</i>
7	Glycerogalactolipids G	Linolenoyl	Linolenoyl	H	<i>L. barbarum</i>
8	Glycerogalactolipids H	Linolenoyl	Linoleoyl	H	<i>L. barbarum</i>
9	Glycerogalactolipids I	Palmitoyl	Linolenoyl	H	<i>L. barbarum</i>
10	Glycerogalactolipids J	Palmitoyl	Linoleoyl	H	<i>L. barbarum</i>
11	Glycerogalactolipids K	Palmitoyl	Oleoyl	H	<i>L. barbarum</i>
12	Glycerogalactolipids L	Stearoyl	Linoleoyl	H	<i>L. barbarum</i>
13	Glycerogalactolipids M	Palmitoyl	Linolenoyl	–	<i>L. barbarum</i>
14	Glycerogalactolipids N	Palmitoyl	Linoleoyl	–	<i>L. barbarum</i>
15	Glycerogalactolipids O	Palmitoyl	Oleoyl	–	<i>L. barbarum</i>
16	Glycerogalactolipids P	Linolenoyl	Linolenoyl	–	<i>L. chinense</i>
17	Glycerogalactolipids Q	Linoleoyl	Linolenoyl	–	<i>L. chinense</i>

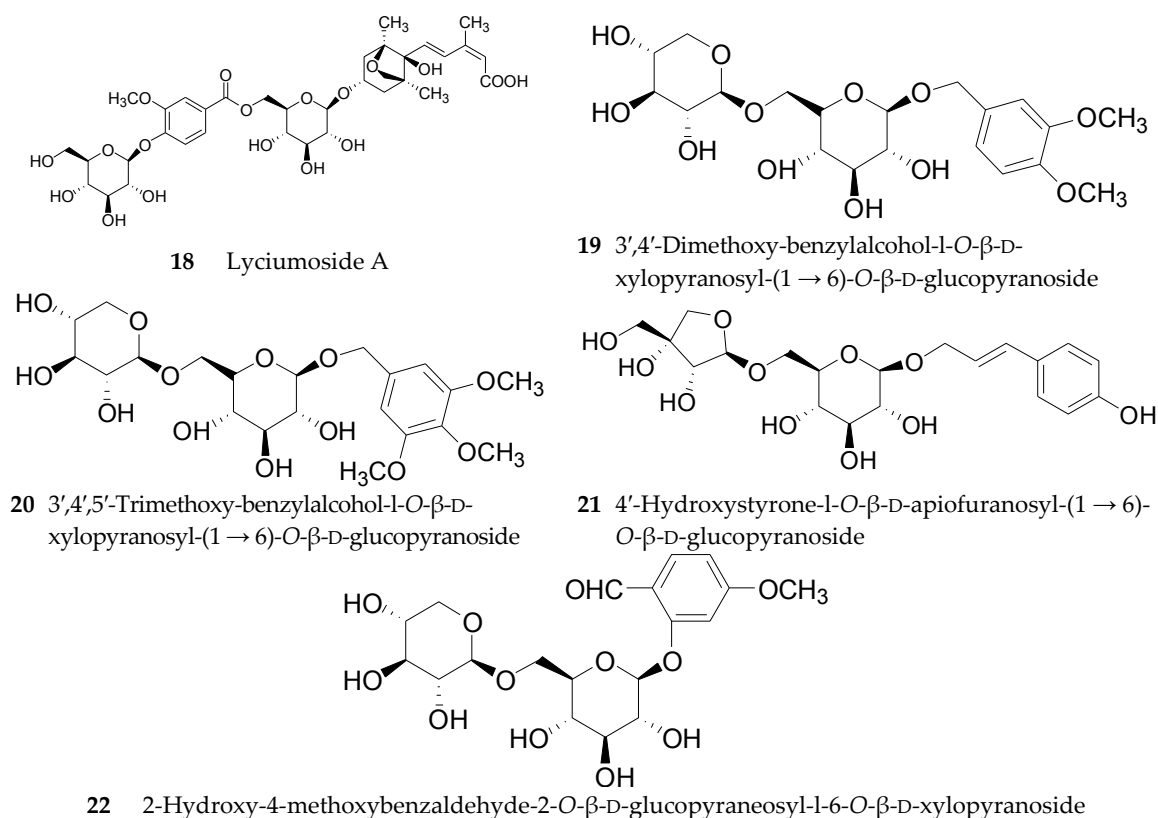


Figure 2. Chemical structures of compounds 18–22.

2.2.2. Phenylpropanoids 23–51

Four phenylpropanoids **23–26**, namely *E*-cinnamic acid (**23**), *E*-ferulic acid (**24**), *E*-coniferol (**25**) and isoscapoletin (**26**) are obtained from wolfberries [56–58]. Four phenylpropanoids, namely scopolin (**27**), fabiatriin (**28**), lyciumin (**29**), and 9-O-(β -D-glucopyranosyl)lyoniresinol (**30**) are obtained from the root bark of *L. chinense* [59–61]. 1-O-Methyl-4-O-*p*-*E*-coumaroyl- α -L-rhamnopyranoside (**31**) is obtained from the fruits of *L. ruthenicum* [62]. The chemical structures of compounds **23–33** are listed in Table 3 and Figure 3. In 2016, 11 phenylpropanoids **32–42** were isolated for the first time by Zhou et al. from *Lycium* [56], including 1-O-*E*-feruloyl-6-O- β -D-xylopyranosyl- β -D-glucopyranoside (**32**), 6-O-*E*-feruloyl-2-O- β -D-glucopyranosyl- α -D-glucopyranoside (**33**), 1-O-*E*-feruloyl- β -D-glucopyranoside (**34**), ethyl-4-O- β -D-glucopyranosyl-*E*-ferulate (**35**), ethyl *E*-ferulate (**36**), *E*-sinapinic acid (**37**), syringenin (**38**), *Z*-ferulic acid (**39**), phloretic acid (**40**), dihydroferulic acid (**41**), and ethyl dihydroferulate (**42**), along with the nine new lycibarbarphenylpropanoids A–I (compounds **43–51**) listed in Table 4.

Table 3. Chemical structures of compounds 26–28.

26–28				
No.	Compounds	R ₁ (R)	R ₂	Source
26	Isoscapoletin	OCH ₃	OH	<i>L. barbarum</i>
27	Scopolin	O- β -D-Glc	OCH ₃	<i>L. chinense</i>
28	Fabiatriin	O- β -D-Glc- β -D-Xyl	OCH ₃	<i>L. chinense</i>

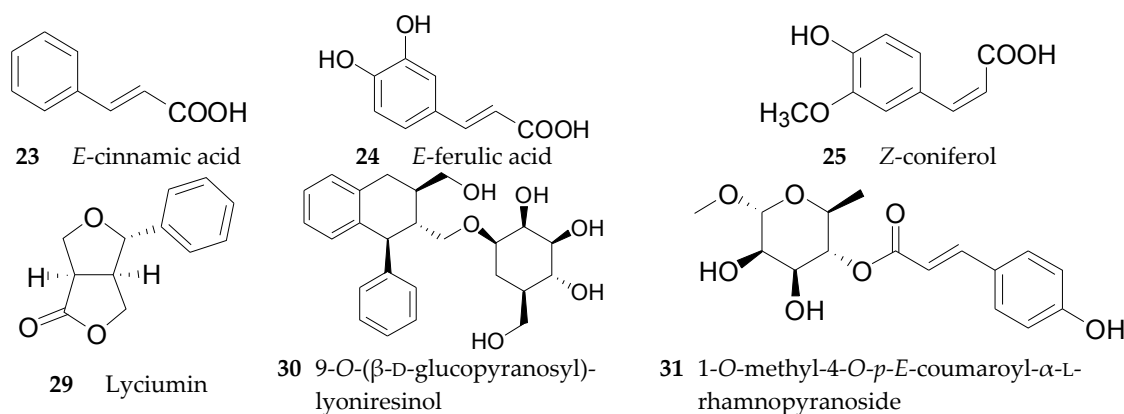
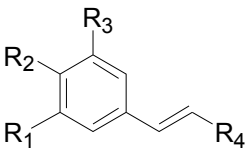
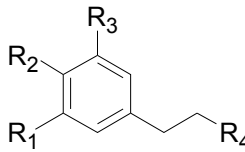


Figure 3. Chemical structures of compounds 23–25, 29–31.

Table 4. Chemical structures of compounds 32–51.

						
32–39, 43–51		40–42				
No.	Compounds	R ₁	R ₂	R ₃	R ₄	Source
32	1- <i>O-E</i> -feruloyl-6- <i>O-β-D</i> -xylopyranosyl-β- <i>D</i> -glucopyranoside	OCH ₃	OH	H	COO-β- <i>D</i> -Glc ⁶ -β- <i>D</i> -Xyl	<i>L. barbarum</i>
33	6- <i>O-E</i> -feruloyl-2- <i>O-β-D</i> -glucopyranosyl-α- <i>D</i> -glucopyranoside	OCH ₃	OH	H	COO ⁶ -α- <i>D</i> -Glc ² -β- <i>D</i> -Glc	<i>L. barbarum</i>
34	1- <i>O-E</i> -feruloyl-β- <i>D</i> -glucopyranoside	OCH ₃	OH	H	COO-β- <i>D</i> -Glc	<i>L. barbarum</i>
35	Ethyl 4- <i>O-β-D</i> -glucopyranosyl- <i>E</i> -ferulate	OCH ₃	<i>O-β-D</i> -Glc	H	COOCH ₂ CH ₃	<i>L. barbarum</i>
36	Ethyl <i>E</i> -ferulate	OCH ₃	OH	H	COOCH ₂ CH ₃	<i>L. barbarum</i>
37	<i>E</i> -sinapinic acid	OCH ₃	OH	OCH ₃	COOH	<i>L. barbarum</i>
38	Syringenin	OCH ₃	OH	OCH ₃	CH ₂ OH	<i>L. barbarum</i>
39	<i>E</i> -ferulic acid	OCH ₃	OH	H	COOH	<i>L. barbarum</i>
40	Phloretic acid	H	OH	H	COOH	<i>L. barbarum</i>
41	Dihydroferulic acid	OCH ₃	OH	H	COOH	<i>L. barbarum</i>
42	Ethyl dihydroferulate	OCH ₃	OH	H	COOCH ₂ CH ₃	<i>L. barbarum</i>
43	Lycibarbarphenylpropanoids A	H	OH	H	COO-β- <i>D</i> -Glc ³ -β- <i>D</i> -Glc	<i>L. barbarum</i>
44	Lycibarbarphenylpropanoids B	H	OH	H	COO-β- <i>D</i> -Glc ⁴ -β- <i>D</i> -Glc	<i>L. barbarum</i>
45	Lycibarbarphenylpropanoids C	OCH ₃	OH	H	COO-β- <i>D</i> -Glc ³ -β- <i>D</i> -Glc	<i>L. barbarum</i>
46	Lycibarbarphenylpropanoids D	OCH ₃	OH	H	COO-β- <i>D</i> -Glc ⁴ -β- <i>D</i> -Glc	<i>L. barbarum</i>
47	Lycibarbarphenylpropanoids E	OCH ₃	OH	H	CH ₂ O-β- <i>D</i> -Glc ³ -β- <i>D</i> -Glc	<i>L. barbarum</i>
48	Lycibarbarphenylpropanoids F	H	<i>O-β-D</i> -Glc ³ -β- <i>D</i> -Glc	H	COOCH ₂ CH ₃	<i>L. barbarum</i>
49	Lycibarbarphenylpropanoids G	H	<i>O-β-D</i> -Glc ⁴ -β- <i>D</i> -Glc	H	COOCH ₂ CH ₃	<i>L. barbarum</i>
50	Lycibarbarphenylpropanoids H	OCH ₃	<i>O-β-D</i> -Glc ⁴ -β- <i>D</i> -Glc	H	COOCH ₂ CH ₃	<i>L. barbarum</i>
51	Lycibarbarphenylpropanoids I	<i>O-β-D</i> -Glc	OH	H	COOCH ₂ CH ₃	<i>L. barbarum</i>

2.2.3. Coumarins 52–61

Nine coumarins, namely *E*-*p*-coumaric acid (52), *Z*-*p*-coumaric acid (53), esculetin (54), fabiatriin (55), scopolin (56), and scopoletin (57), have been reported, and three new coumarins, 6-*O*-*E*-*p*-coumaroyl-2-*O*-β-*D*-glucopyranosyl-α-*D*-glucopyranoside (58), ethyl 4-*O*-β-*D*-glucopyranosyl-*E*-*p*-coumarate (59), ethyl *E*-*p*-coumarate (60) and lycibarbarcoumarin A (61), have been obtained from the fruits of *L. barbarum* in 2016 [56]. Compounds 55 and 56 were isolated from the root bark and

fruits of *L. chinense* [61], while **52–54** and **57** were isolated from the fruits of *L. barbarum* [63]. The chemical structures of these coumarins are listed in Figure 4 and Table 5.

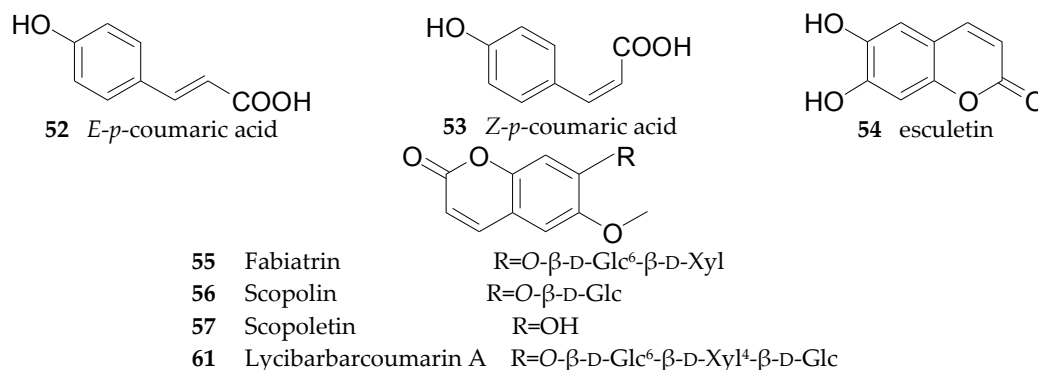


Figure 4. Chemical structures of compounds **52–57**, **61**.

Table 5. Chemical structures of compounds **58–60**.

No.	Compounds	R ₁	R ₂	R ₃	R ₄	Source
58	6- <i>O-E-p</i> -coumaroyl-2- <i>O</i> - β -D-glucopyranosyl- α -D-glucopyranoside	H	OH	H	COO ⁶ - α -D-Glc ² - β -D-Glc	<i>L. barbarum</i>
59	Ethyl-4- <i>O</i> - β -D-glucopyranosyl- <i>E-p</i> -coumarate	H	<i>O</i> - β -D-Glc	H	COOCH ₂ CH ₃	<i>L. barbarum</i>
60	Ethyl <i>E-p</i> -coumarate	H	OH	H	COOCH ₂ CH ₃	<i>L. barbarum</i>

2.2.4. Lignans **62–74**

Eight lignans, including pinoresinol (**62**), arctigenin (**63**), arctiin (**64**), medioresinol (**65**), syringaresinol (**66**), 4-*O*-(β -D-glucopyranosyl)syringaresinol (**67**), *threo*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (**68**), and *erythro*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (**69**), have been isolated from the fruits of *L. barbarum* [56]. (β)-Lyoniresinol 3-*O*- β -D-glucopyranoside (**70**), lyciumlignan A (**71**), lyciumlignan B (**72**), lyciumlignan C (**73**), and (7*R*,8*S*)-4,9,9'-trihydroxy-3,3'-dimethoxy-7'-en-8,4'-oxyneolignan-7-*O*- β -D-glucopyranoside (**74**) were obtained from the root bark of *L. chinense* [54,60,64]. Among them, **65–70** were first isolated from the fruits of *L. barbarum* in 2016 [56]. The chemical structures of these lignans are listed in Figure 5 and Table 6.

Table 6. Chemical structures of compounds **62** and **65–67**.

No.	Compounds	R ₁	R ₂	R ₃	Source
62	Pinoresinol	H	OH	H	<i>L. barbarum</i>
65	Medioresinol	H	OH	OCH ₃	<i>L. barbarum</i>
66	Syringaresinol	OCH ₃	OH	OCH ₃	<i>L. barbarum</i>
67	4- <i>O</i> -(β -D-glucopyranosyl)syringaresinol	OCH ₃	<i>O</i> - β -D-Glc	OCH ₃	<i>L. barbarum</i>

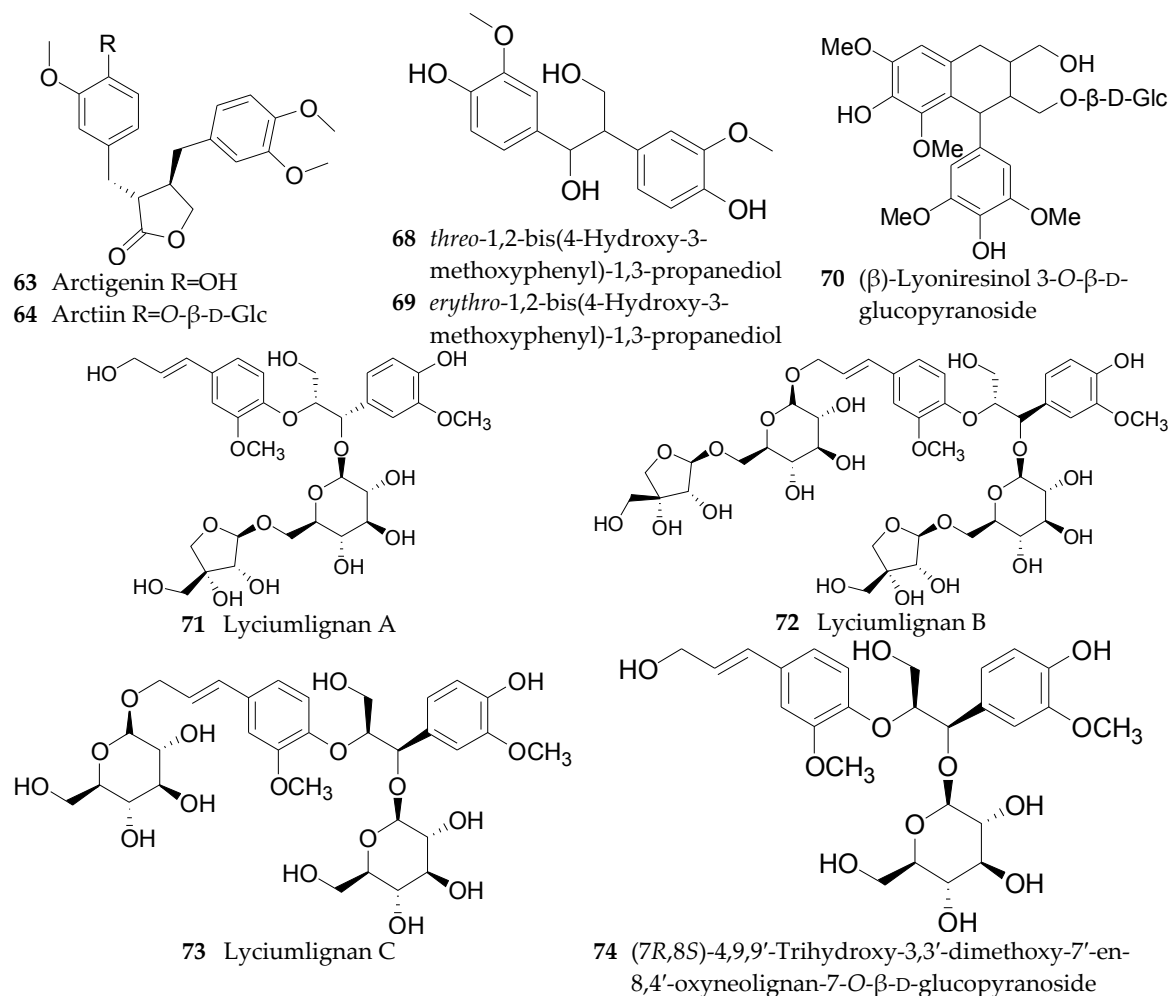


Figure 5. Chemical structures of compounds 63–64 and 68–74.

2.2.5. Flavonoids 75–106

Twenty-seven flavonoids **75–101** have been reported from the genus *Lycium*, are listed in Tables 7 and 8 and Figures 6 and 7. Compound **75** was isolated from the flowers of *L. barbarum* [58], while **76–83** were identified from the fruits of *L. barbarum* [62,65–69]. Compound **84** was isolated from the fruits of *L. chinense* [70], whereas **85–91** were isolated from the leaves of *L. chinense* [62,66,68,71]. Compound **92** and **93** were isolated from the leaves of *L. halimifolium* [72]. Compounds **94–98** were isolated from the fruits of *L. ruthenicum* [16,62]. Compounds **99–101** were isolated from the root bark of *L. chinense* [54,73,74]. Additionally, Zhou et al. isolated five isoflavonoids, namely derrone (**102**), alpinumisoflavone (**103**), auricularin (**104**), maackianin (**105**) and maackianin (**106**) from the fruits of *L. barbarum* [56,75,76].

Table 7. Chemical structures of compounds 75–80, 82–83, 85–87 and 89–93.

 75,77–80,87,90,92 78,82–83,93 85–86,89,91					
No.	Compounds	R ₁	R ₂	R ₃	Source
75	Quercitrin	OH	OH	O-α-L-Rha	<i>L. barbarum</i>
76	Kaempferol	OH	OH	–	<i>L. barbarum</i>
77	Quercetin	OH	OH	OH	<i>L. barbarum</i>
78	Rutin	OH	OH	O-β-D-Glc ⁶ -α-L-Rha	<i>L. barbarum</i>
79	Narcissoside	OH	OCH ₃	O-β-D-Glc ⁶ -α-L-Rha	<i>L. barbarum</i>

80	7-O-(β-D-Glucopyranosyl)-rutin	O-β-D-Glc	OH	O-β-D-Glc ⁶ -α-L-Rha	<i>L. barbarum</i>
82	7-O-(β-D-Glucopyranosyl)-nicotiflorin	O-β-D-Glc	O-β-D-Glc ⁶ -α-L-Rha	–	<i>L. barbarum</i>
83	7-O-(β-D-Glucopyranosyl)-3-O-[β-D-glucopyranosyl]-(1 → 2)-β-D-galactop	O-β-D-Glc	O-β-D-Glc ⁶ -α-L-Glc	–	<i>L. barbarum</i>
85	Luteolin	OH	OH	OH	<i>L. chinense</i>
86	Acacetin	OH	H	OCH ₃	<i>L. chinense</i>
87	7-O-(β-D-Glucopyranosyl)-3-O-[β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl]-quercetin	O-β-D-Glc	OH	O-β-D-Glc ² -β-D-Glc	<i>L. chinense</i>
89	7-O-[α-L-Rhamno-pyranosyl-(1 → 6)-β-D-glucopyranosyl]-acacetin	O-β-D-Glc ⁶ -α-L-Rha	H	OCH ₃	<i>L. chinense</i>
90	3-O-Sophoroside-quercetin	OH	OH	O-β-D-Glc ² -β-D-Glc	<i>L. chinense</i>
91	Apigenin	OH	H	OH	<i>L. chinense</i>
92	Isoquercitrin	OH	OH	O-β-D-Glc	<i>L. halimifolium</i>
93	Nicotiflorin	OH	O-β-D-Glc ⁶ -α-L-Rha	–	<i>L. halimifolium</i>

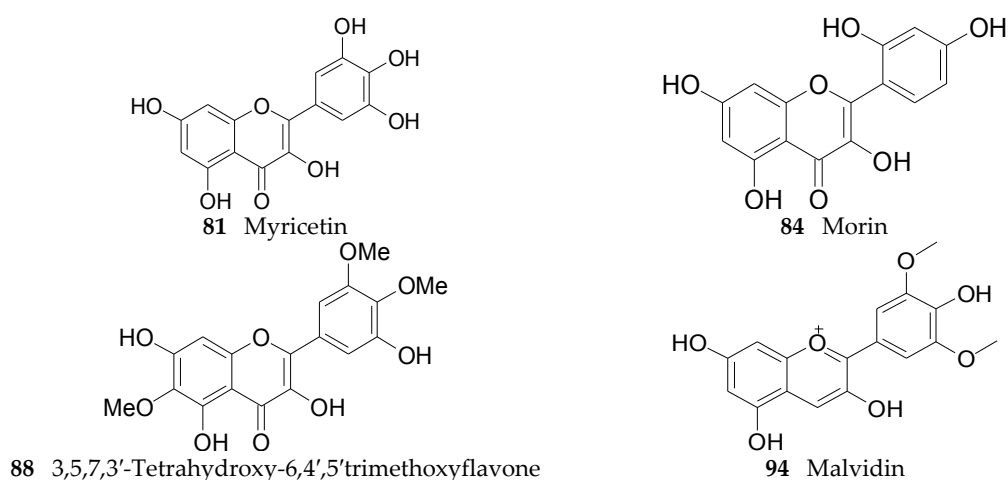


Figure 6. Chemical structures of compounds 81, 84, 88 and 94.

Table 8. Chemical structures of compounds 95–98.

No.	Compounds	R ₁	R ₂	Source
95	5-O-(β-D-Glucopyranosyl)-3-O-[4-O- <i>p</i> -E-coumaroyl-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl]-peonidin	H	OH	<i>L. ruthenicum</i>
96	5-O-(β-D-Glucopyranosyl)-3-O-[4-O- <i>p</i> -E-coumaroyl-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl]-petunidin	OH	OH	<i>L. ruthenicum</i>

97	5-O-(β -D-Glucopyranosyl)-3-O-[4-O- <i>p</i> -Z-coumaroyl- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl]-malvidin	OCH ₃	OH	<i>L. ruthenicum</i>
98	5-O-(β -D-Glucopyranosyl)-3-O-[4-O- <i>p</i> -E-(β -D-glucopyranoside)-coumaroyl- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl]-petunidin	OH	O- β -D-Glc	<i>L. ruthenicum</i>

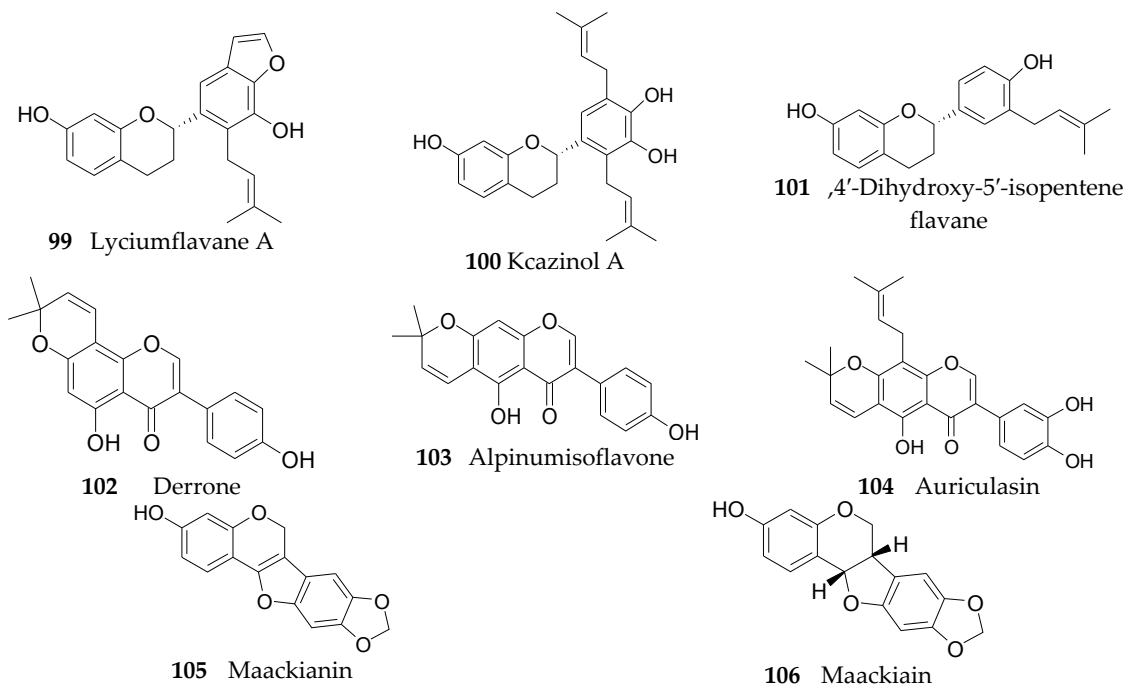
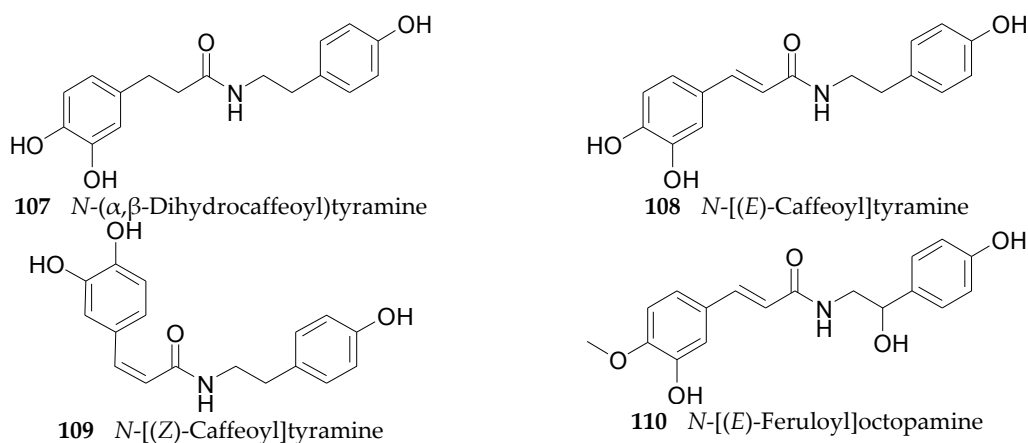
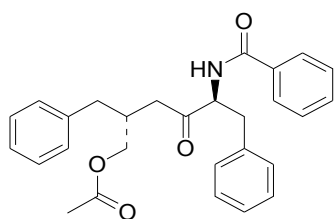
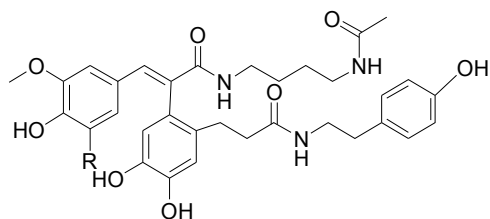
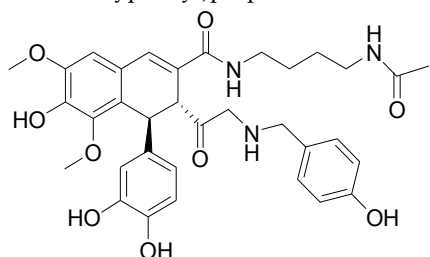
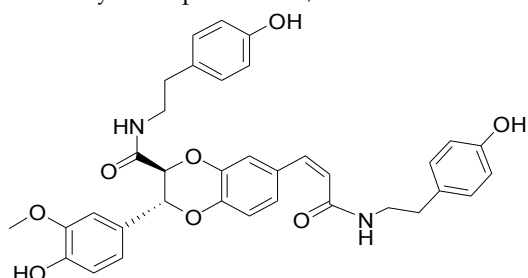
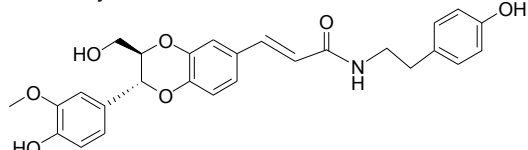
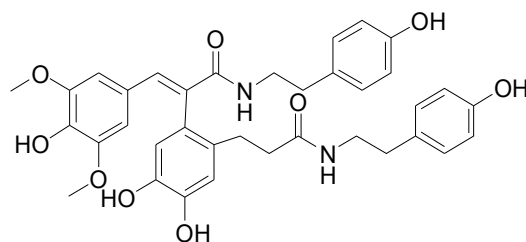
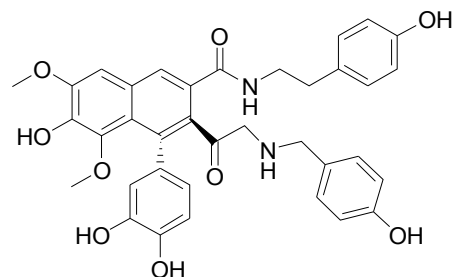
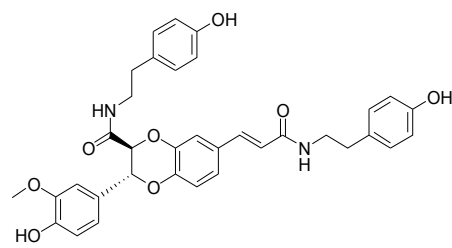
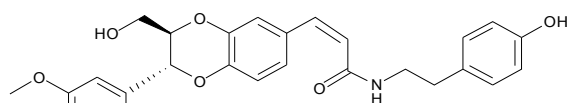
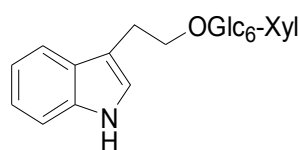


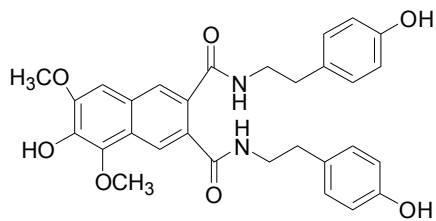
Figure 7. Chemical structures of compounds 99–106.

2.2.6. Amides 107–143

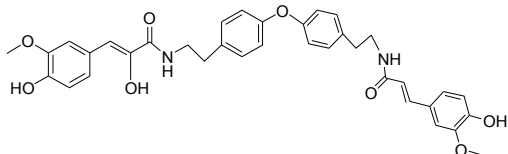
Sixteen amides **107–122** have been isolated from the root bark of *L. chinense* [9,54,60,77–80], 19 amides (**123–141**) have been isolated from the fruits of *L. barbarum* [81–88]. Meanwhile, two cerebrosides **142** and **143** have been obtained from fruits of *L. chinense* [89]. The chemical structures of these amides are shown in Figure 8.



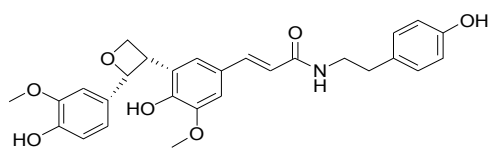
**111** Aurantiamide acetate**113** (*E*)-*N*-(4-Acetamidobutyl)-2-[4,5-dihydroxy-2-[3-[2-(4-hydroxyphenyl)ethylamino]-3-oxopropyl]-phenyl]-3-(4-hydroxy-3-methoxyphenyl)prop-2-enamide R=H**114** (*E*)-*N*-(4-Acetamidobutyl)-2-[4,5-dihydroxy-2-[3-[2-(4-hydroxyphenyl)ethylamino]-3-oxopropyl]-phenyl]-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enamide R=OCH₃**116** (1*S*,2*R*)-*N*3-(4-Acetamidobutyl)-1-(3,4-dihydroxy-phenyl)-7-hydroxy-*N*2-(4-hydroxyphenethyl)-6,8-dimethoxy-1,2-dihydro-naphthalene-2,3-dicarboxamide**118** (2,3-*E*)-3-(3-hydroxy-5-methoxyphenyl)-*N*-(4-hydroxyphenethyl)-7-((*Z*)-3-[(4-hydroxyphenethyl)amino]-3-oxoprop-1-en-1-yl)-2,3-dihydrobenzo[*b*][1,4]dioxine-2-carboxamide**120** (*E*)-3-((2,3-*E*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-*N*-(4-hydroxyphenethyl)-acrylamide**112** (*E*)-2-[4,5-Dihydroxy-2-[3-[2-(4-hydroxyphenyl)ethylamino]-3-oxopropyl]-phenyl]-3-(4-hydroxy-3,5-dimethoxyphenyl)-*N*-[2-(4-hydroxyphenyl)ethyl]prop-2-enamide**115** (1*R*,2*S*)-1-(3,4-Dihydroxyphenyl)-7-hydroxy-*N*2,*N*3-bis(4-hydroxyphenethyl)-6,8-dimethoxy-1,2-dihydro-naphthalene-2,3-dicarboxamide**117** (2,3-*E*)-3-(3-Hydroxy-5-methoxyphenyl)-*N*-(4-hydroxyphenethyl)-7-((*E*)-3-[(4-hydroxyphenethyl)=amino]-3-oxoprop-1-en-1-yl)-2,3-dihydrobenzo[*b*][1,4]dioxine-2-carboxamide**119** (*Z*)-3-((2,3-*E*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-*N*-(4-hydroxyphenethyl)acrylamide**121** Indole glycoside



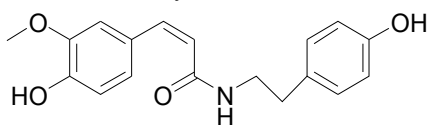
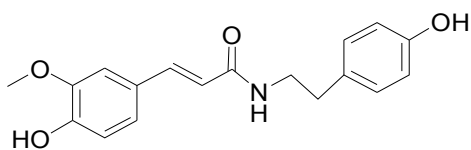
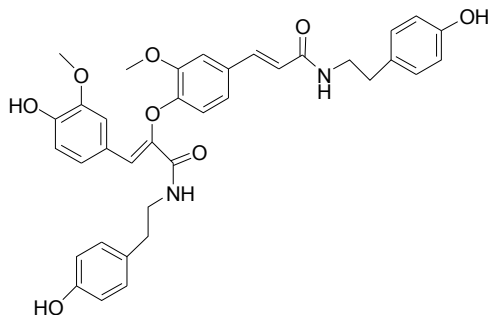
122 Lyciumamide D



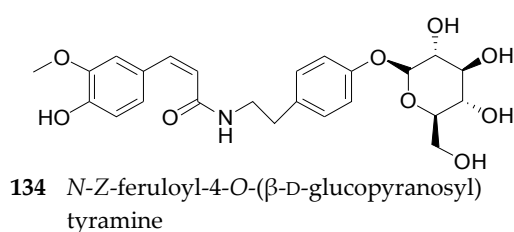
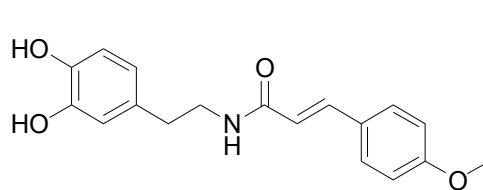
124 Lyciumamide A



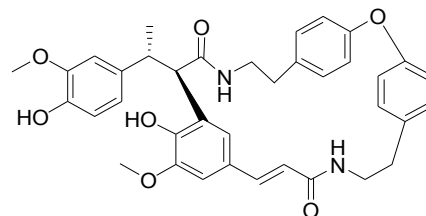
126 Lyciumamide C

128 *N-Z*-feruloyl-tyramine130 *N-E*-feruloyl-tyramine

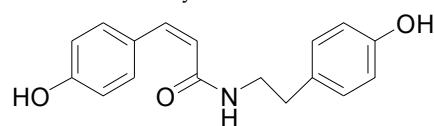
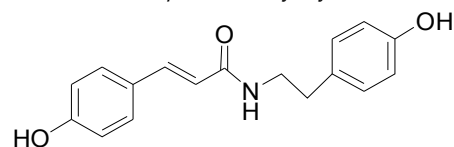
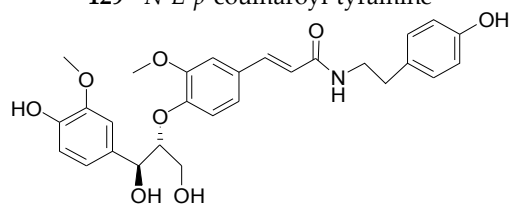
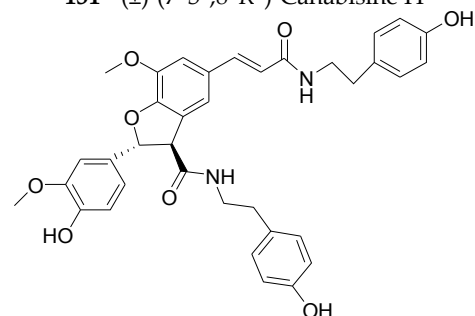
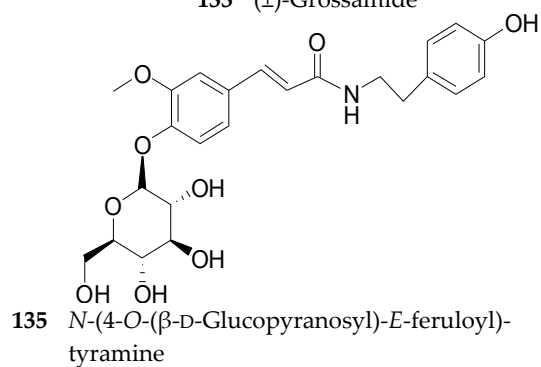
132 Cannabisin F

134 *N-Z*-feruloyl-4-*O*-(β -D-glucopyranosyl) tyramine

123 Lyciumide A



125 Lyciumamide B

127 *N-Z*-*p*-coumaroyl-tyramine129 *N-E*-*p*-coumaroyl-tyramine131 ($\pm$)-(7''*S**,8''*R**)-Cannabisine H133 ($\pm$)-Grossamide135 *N*-(4-*O*-(β -D-Glucopyranosyl)-*E*-feruloyl)-tyramine

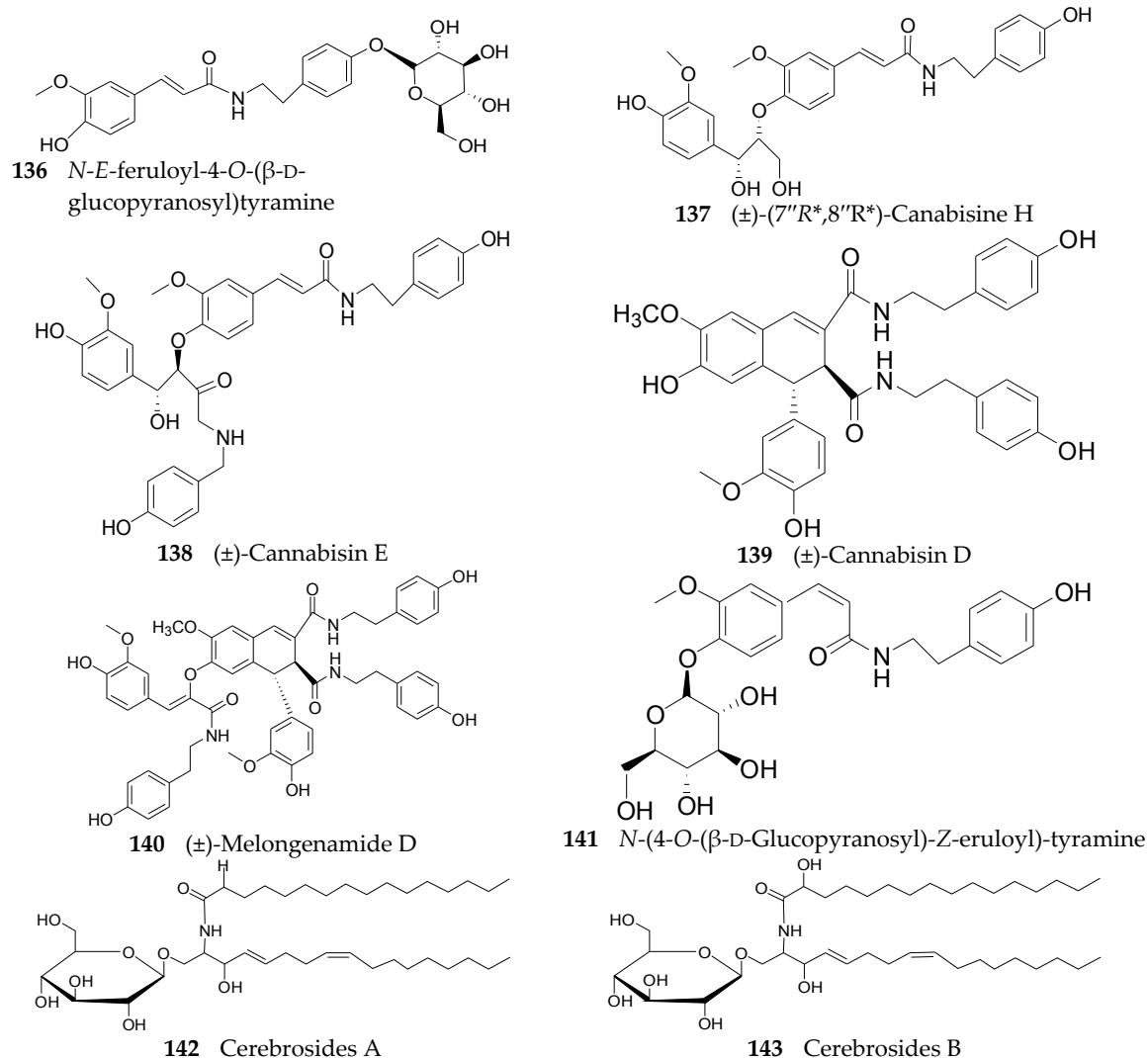


Figure 8. Chemical structures of compounds **107–143**.

2.2.7. Alkaloids **144–215**

To date, 72 alkaloids have been identified, which can be classified into five categories: nortropane, imidazole, piperidine, pyrrole, spermine, tropane, and other alkaloids.

Nortropane Alkaloids

Fourteen nortropane alkaloids **144–157**, shown in Figure 9, have been isolated from the root bark of *L. chinense* [90].

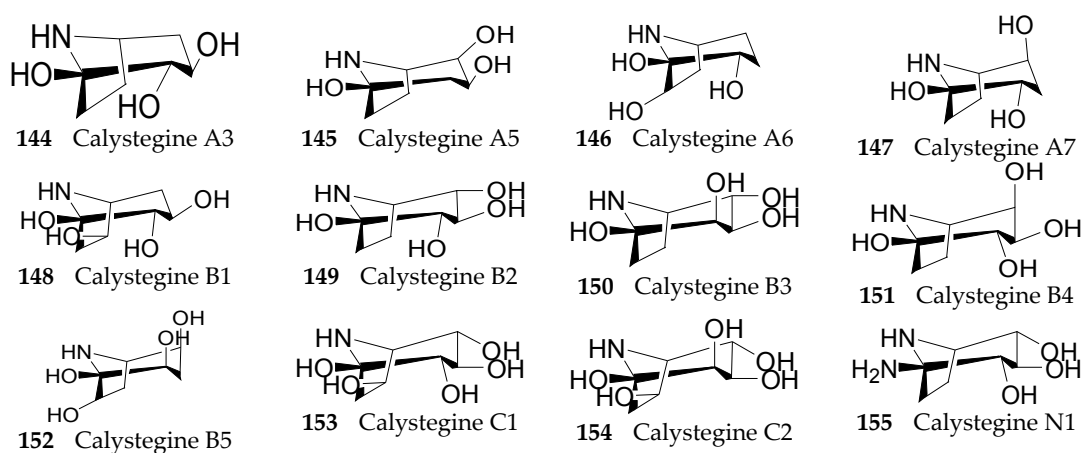




Figure 9. Chemical structures of compounds 144–157.

Imidazole Alkaloids

Six imidazole alkaloids **158–162** were detected in the leaves of *L. cestroides* [91]; Meanwhile, one imidazole, Na-[(*E*)-cinnamoyl]histamine (**163**), was obtained from the leaves of *L. barbarum* [66], listed in Figure 10.

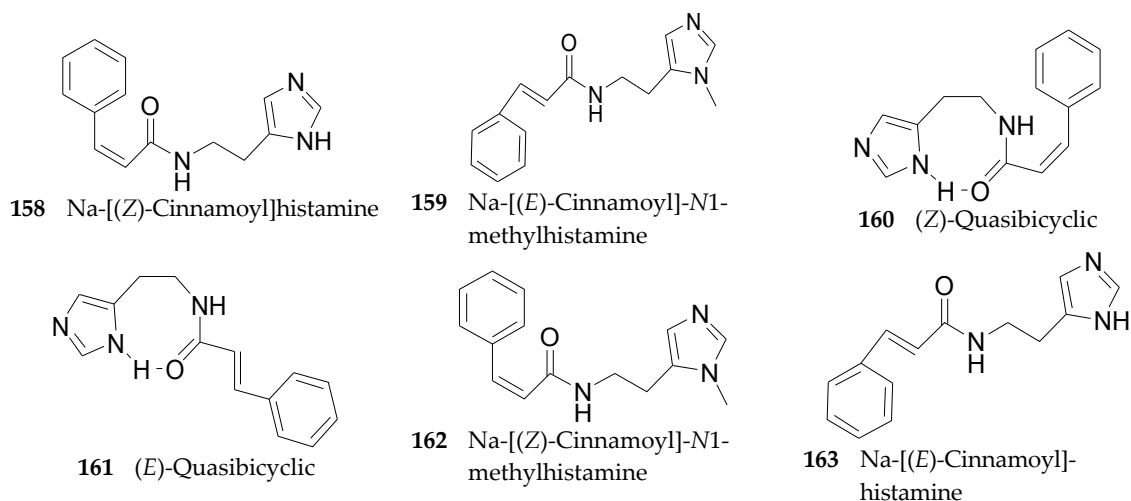


Figure 10. Chemical structures of compounds 158–163.

Piperidine Alkaloids

5-hydroxy-2-pyridylmethyl ketone (**164**), methyl 5-hydroxy-2-pyridinecarboxylate (**165**), fagomine (**166**), and 6-deoxyfagomine (**167**), listed in Figure 11, have been isolated and identified from the genus *Lycium*; among them. Compounds **164** and **165** are from the fruits of *L. barbarum* [92], and **166** and **167** are from the root bark of *L. chinense* [90].

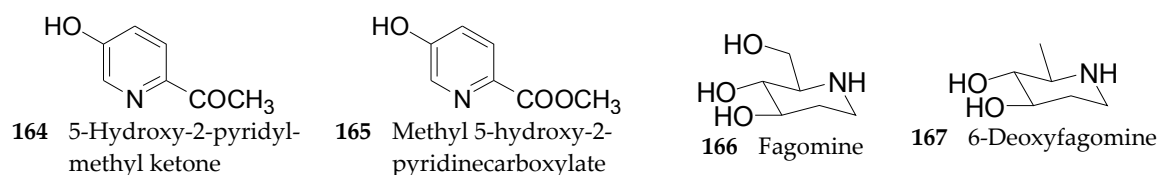


Figure 11. Chemical structures of compounds 164–167.

Pyrrole Alkaloids

Thirteen pyrrole alkaloids **168–180** have been isolated from the fruits of *L. chinense* [93–95]. Likewise, 2-formyl-5-hydroxymethylpyrrole (**181**) and 2-formyl-5-methoxymethylpyrrole (**182**) were isolated from the fruits of *L. barbarum* [92]. Two pyrrolidine alkaloids, alkaloid I (**183**) and alkaloid II (**184**), are obtained from the root bark of *L. chinense* [96]. The chemical structures of these pyrrole alkaloids are listed in Figure 12.

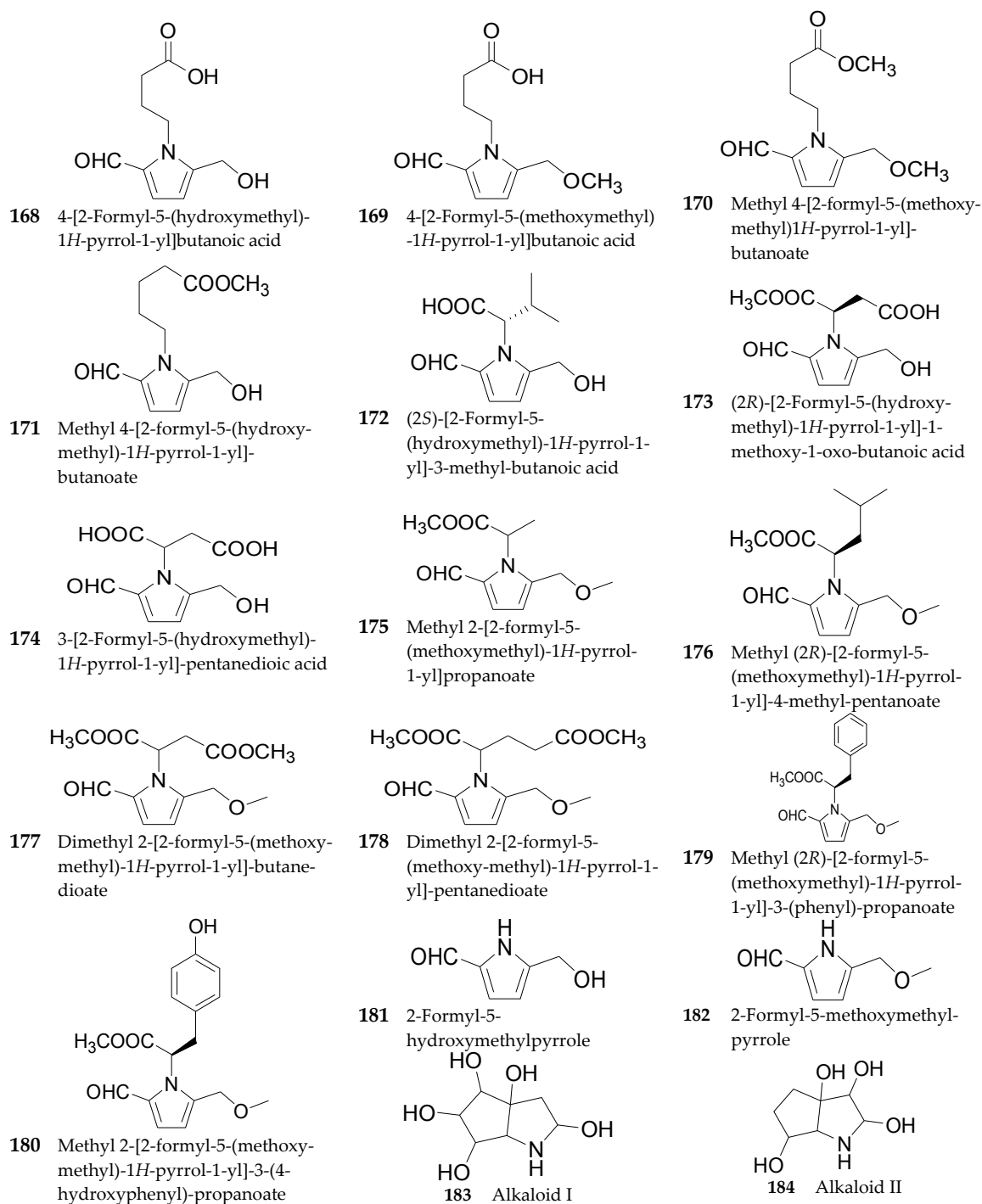
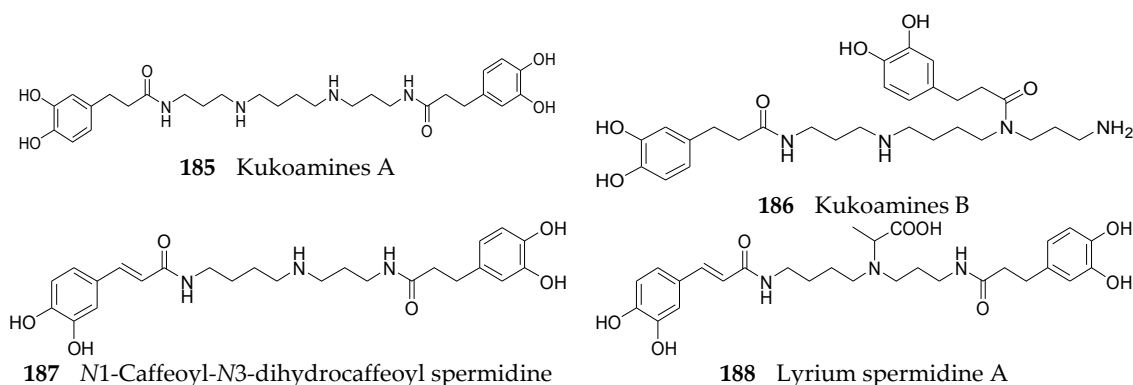


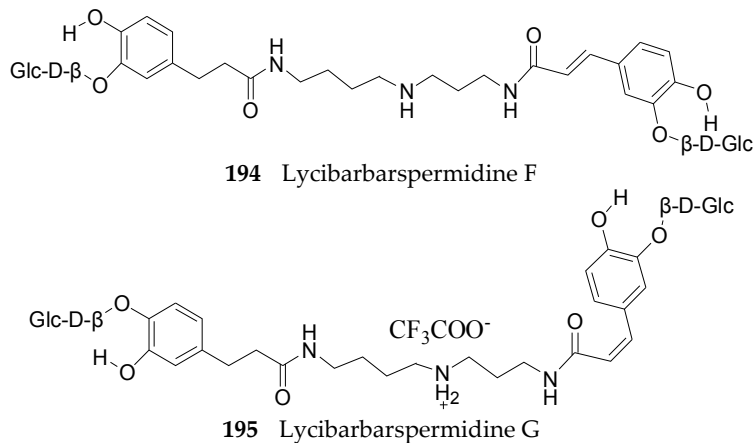
Figure 12. Chemical structures of compounds 168–184.

Spermine Alkaloids

Nineteen spermine alkaloids have been found in the genus *Lycium*. Kukoamines A (**185**) and kukoamines B (**186**) are from the root bark of *L. chinense* [97,98], while *N*1-caffeoyl-*N*3-dihydrocaffeoyl spermidine (**187**) and lyrium spermidine A (**188**) are from the fruits of *L. ruthenicum* [62,99], listed in Figure 13. Another 15 spermine alkaloids, lycibarbarspermidine A–O (**189–203**), listed in Tables 9 and 10 and Figures 13–15, are from *L. barbarum* [100].

**Figure 13.** Chemical structures of compounds 185–188.**Table 9.** Chemical structures of compounds 189–193.

189–193						
No.	Compounds	R ₁	R ₂	R ₃	R ₄	Source
189	Lycibarbarspermidine A	H	β-D-Glc	H	H	<i>L. barbarum</i>
190	Lycibarbarspermidine B	H	H	β-D-Glc	H	<i>L. barbarum</i>
191	Lycibarbarspermidine C	β-D-Glc	H	H	H	<i>L. barbarum</i>
192	Lycibarbarspermidine D	H	H	H	β-D-Glc	<i>L. barbarum</i>
193	Lycibarbarspermidine E	H	β-D-Glc	β-D-Glc	H	<i>L. barbarum</i>

**Figure 14.** Chemical structures of compounds 194 and 195.**Table 10.** Chemical structures of compounds 196–200.

196–200						
No.	Compounds	R ₁	R ₂	R ₃	R ₄	Source
196	Lycibarbarspermidine H	H	H	H	β-D-Glc	<i>L. barbarum</i>
197	Lycibarbarspermidine I	H	β-D-Glc	H	H	<i>L. barbarum</i>
198	Lycibarbarspermidine J	H	H	β-D-Glc	H	<i>L. barbarum</i>
199	Lycibarbarspermidine K	β-D-Glc	H	β-D-Glc	H	<i>L. barbarum</i>
200	Lycibarbarspermidine L	H	β-D-Glc	H	β-D-Glc	<i>L. barbarum</i>

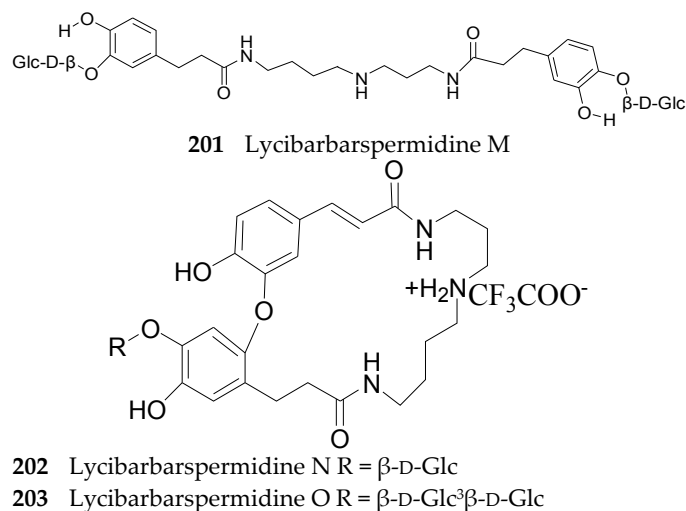
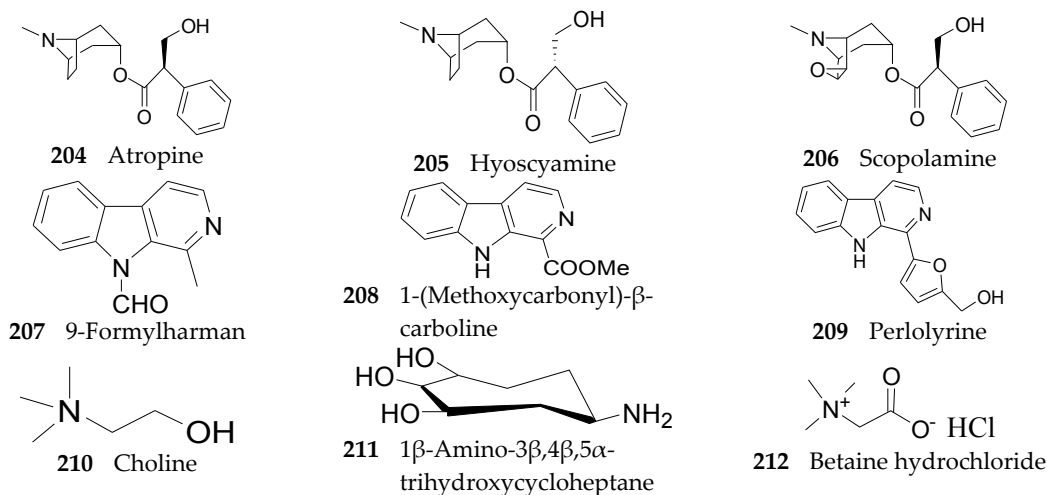


Figure 15. Chemical structures of compounds 201–203.

Tropane Alkaloids

As we know, the genus *Lycium* has been used as both a medicine and a food for a long time in Asia, particularly in China. However, the safety of *Lycium* has been questioned for some time, especially after the detection of the three tropane alkaloids atropine (204), hyoscyamine (205), and scopolamine (206) [101]. Atropine and hyoscyamine were identified from the fruits of *L. barbarum* gathered in India, while scopolamine was identified from *L. halimifolium* at concentrations higher than the toxic dose. However, another scholar, seeking to verify these reports, demonstrated that the atropine content of *L. barbarum* from different sources was just 3.0 ppb—far below the poisoning dose [102]. It was demonstrated that none of the toxic compounds were detected in fruits, leaves, stems and roots of three *L. barbarum* varieties ('No. 1', 'New Big' and 'Amber Sweet Goji') by densitometric TLC analysis [103]. Through field investigation and model specimen inspections, the above three tropane alkaloids were determined to be from *Lycium europaeum* rather than the *L. barbarum*. Thus, the genus *Lycium* is likely non-toxic, and consumers can rest assured that its use is safe [104].

Other than the alkaloids that have been already mentioned, there are nine others that have been obtained from this genus, including 9-formylharman (207), 1-(methoxycarbonyl)- β -carboline (208), perlolyrine (209), choline (210), 1 β -amino-3 β ,4 β ,5 α -trihydroxycycloheptane (211), betaine hydrochloride (212), nicotianamine (213), betaine (214), and melatonin (215). Compounds 207–209 were isolated from the fruits of *L. chinense* [105], while 210–212 were isolated from the root bark of *L. chinense* [90]. Compound 213 was isolated from the leaves and flowers of *L. chinense* [106], and 214 and 215 were isolated from the fruits of *L. barbarum* [107,108]. The chemical structures of these tropane alkaloids are listed in Figure 16.



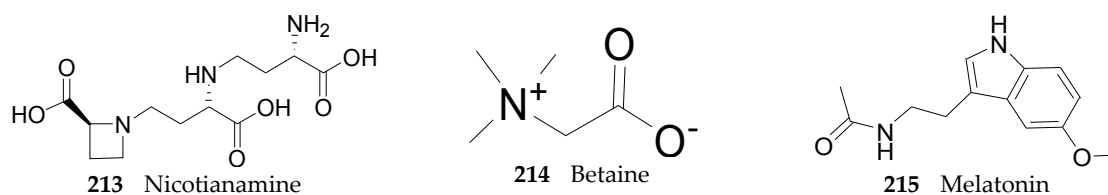


Figure 16. Chemical structures of compounds 204–215.

2.2.8. Anthraquinones 216–219

Four anthraquinones: emodin (216), physcion (217), 6-hydroxyrubiadin (218), and 3-*O*-(2-*O*- α -L-rhamnopyranosyl-6-*O*-acetyl- β -D-glucopyranosyl)-6-hydroxy-rubiadin (219), listed in Figure 17, have been obtained from the root bark of *L. chinense* [61,109].

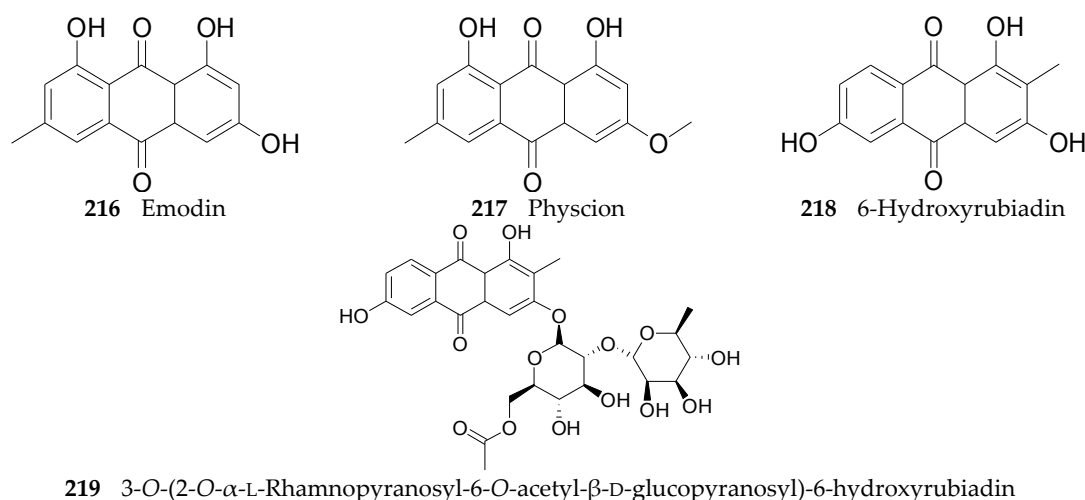
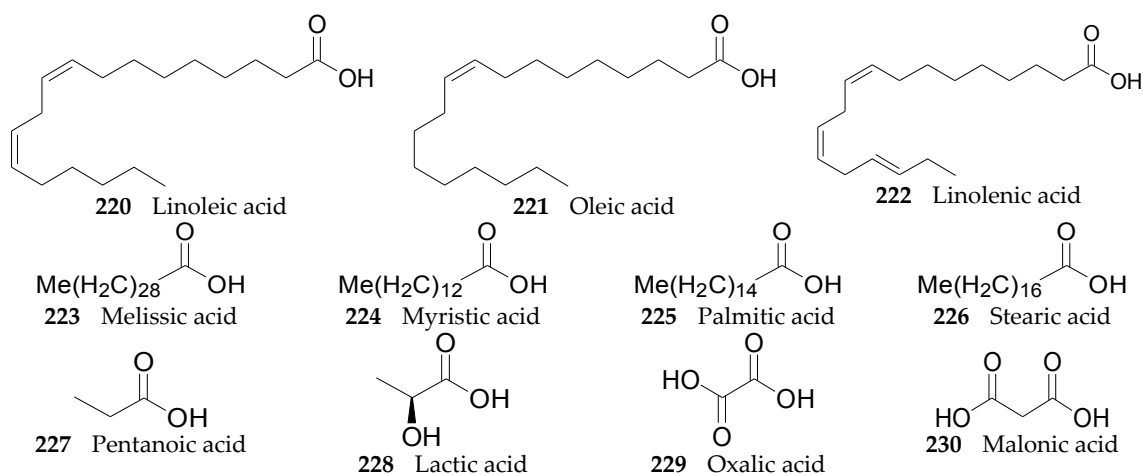


Figure 17. Chemical structures of compounds 216–219.

2.2.9. Organic Acids 220–251

To this point, 32 organic acids, listed in Figure 18, have been identified from the genus *Lycium*, which can be classified into two groups: aliphatic acids 220–238 and aromatic acids and their derivatives 239–251. Compounds 220–225 and 240–244 were isolated from the fruits of *L. barbarum* [56,63,65,107,110–112]; 239 and 245 were isolated from the leaves of *L. barbarum* [66]; 226 was isolated from the root of *L. chinense* [113]; 227, 248 and 249 were isolated from the fruits of *L. chinense* [70,114]; 228–233 and 248 were isolated from the leaves of *L. chinense* [115]; 234, 235, and 249–251 were isolated from the root bark of *L. chinense* [53,78,93,116,117], and 236–238 were isolated from the fruits of *L. urcomanicum* [118,119].



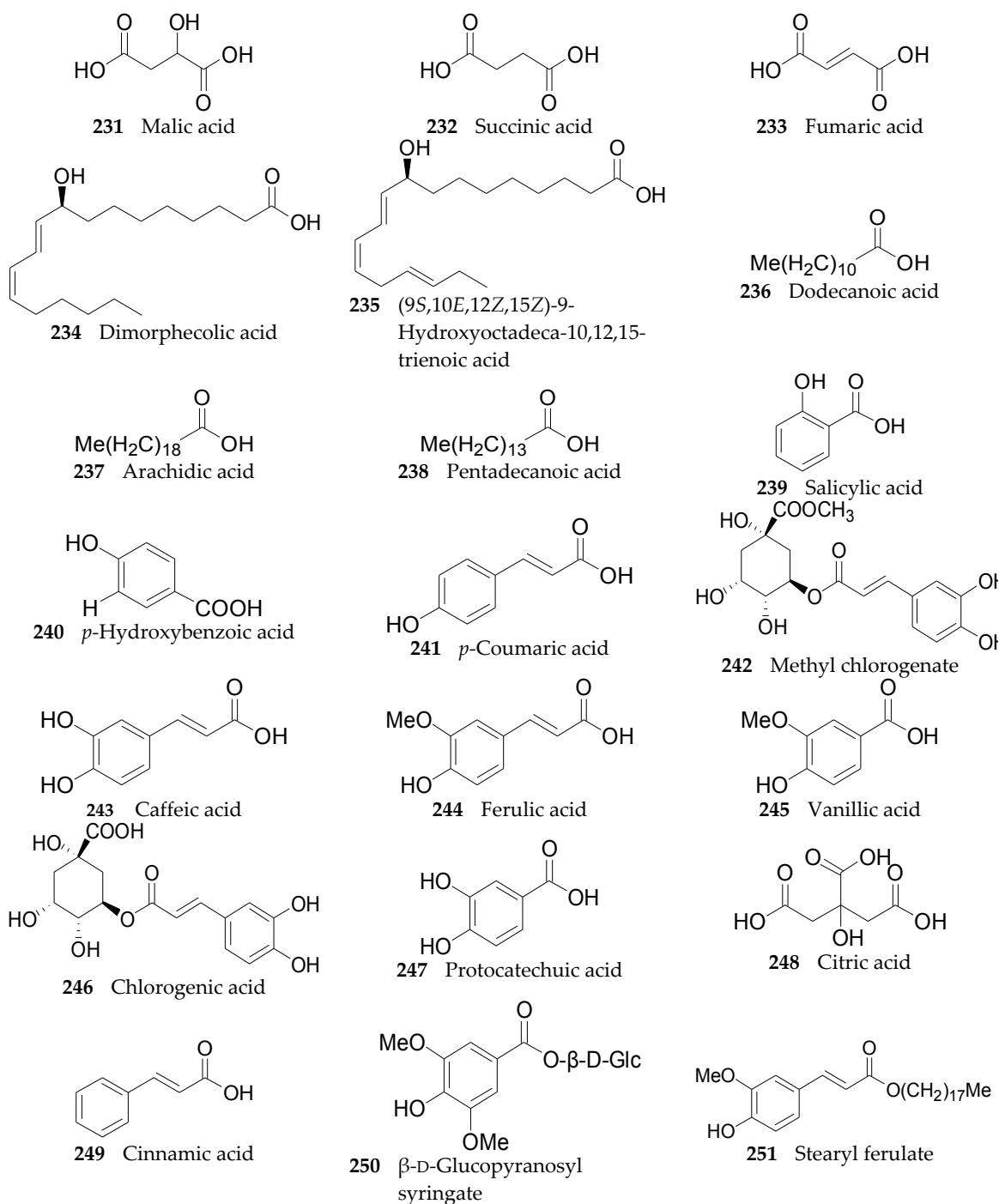


Figure 18. Chemical structures of compounds 220–251.

2.2.10. Terpenoids 252–290

Thirty-seven terpenoids, listed in Figures 19–21 and Tables 11 and 12, have been found in the genus *Lycium*, mainly including monoterpenes 252–256, sesquiterpenes 257–263, diterpenoids 264–274, and carotenoids 275–290. Among them, carotenoids are one of the more important constituents of the *Lycium* fruits. Thus compounds 256 and 275–286 were isolated from the fruits of *L. barbarum* [120–123]; 253, 254, 258, 259 and 287–290 were isolated from the fruits of *L. chinense* [120,121,124–126]; 252 and 264–272 were isolated from the leaves of *L. chinense* [127,128]; 255, 258 and 273–274 were isolated from the root bark of *L. chinense* [80,116]; 260 and 261 were isolated from the leaves of *L. halimifolium* [23]; and 262 and 265 were isolated from the leaves of *L. barbarum* [129].

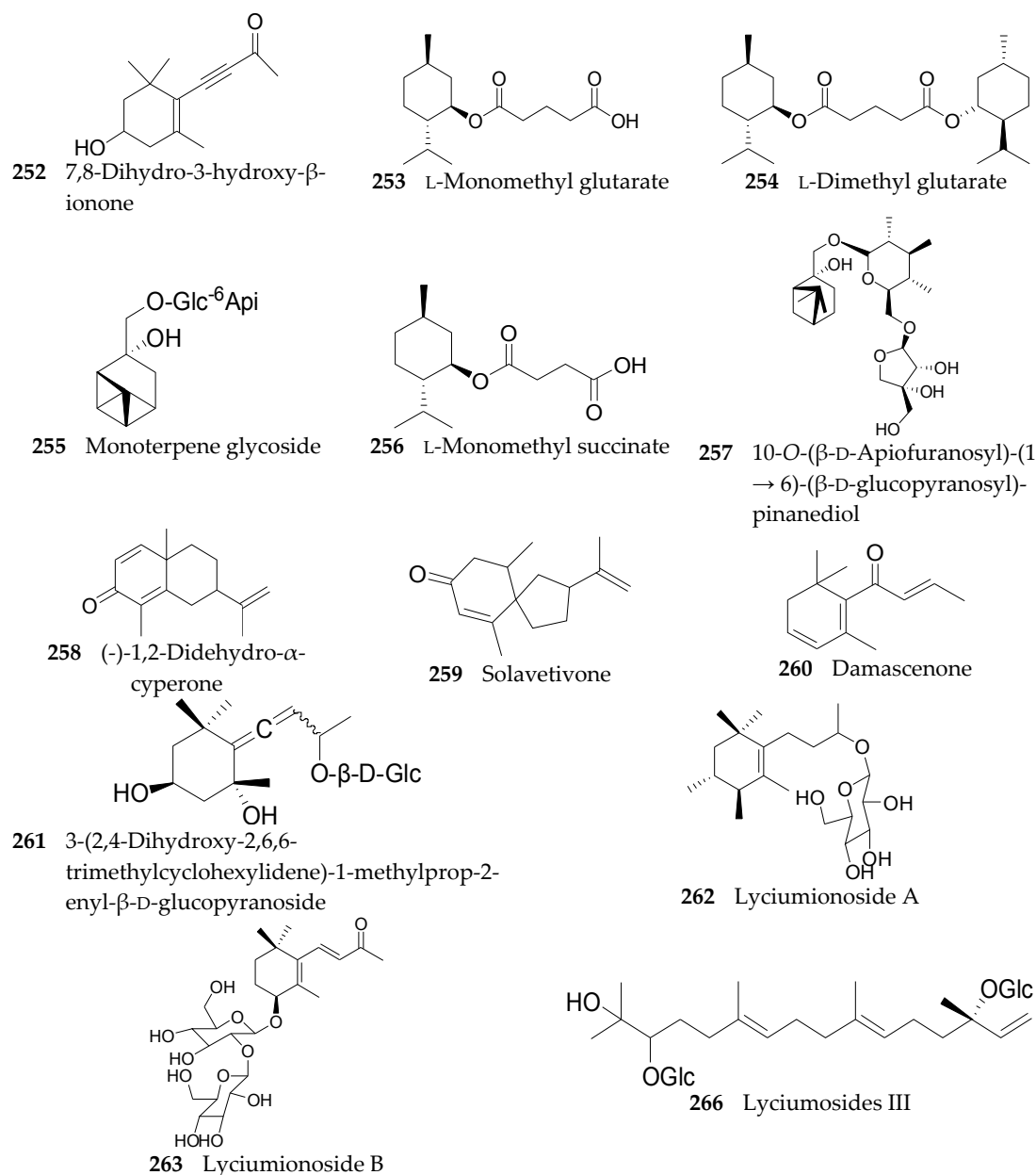
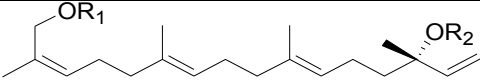


Figure 19. Chemical structures of compounds 252–263, 266.

Table 11. Chemical structures of compounds 264–265, 267–270 and 272–273.

 264–265, 267–270, 272–273				
No.	Compounds	R ₁	R ₂	Source
264	Lyciumosides I	Glc	Glc	<i>L. chinense</i>
265	Lyciumosides II	Glc ² -Glc	Glc	<i>L. chinense</i>
267	Lyciumosides IV	Glc	Glc ⁴ -Rha	<i>L. chinense</i>
268	Lyciumosides V	Glc ⁶ -Rha	Glc	<i>L. chinense</i>
269	Lyciumosides VI	Glc ⁶ -Rha	Glc ⁴ -Rha	<i>L. chinense</i>
270	Lyciumosides VII	Glc ² -Rha(⁶ -Glc)	Glc	<i>L. chinense</i>
272	Lyciumosides IX	Glc	6-O-malonyl-Glc	<i>L. chinense</i>
273	Capsianoside II	Rha ³ -Glc ⁶ -Rha	Glc ² -Glc	<i>L. chinense</i>

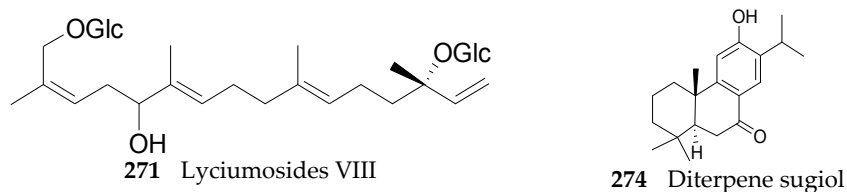
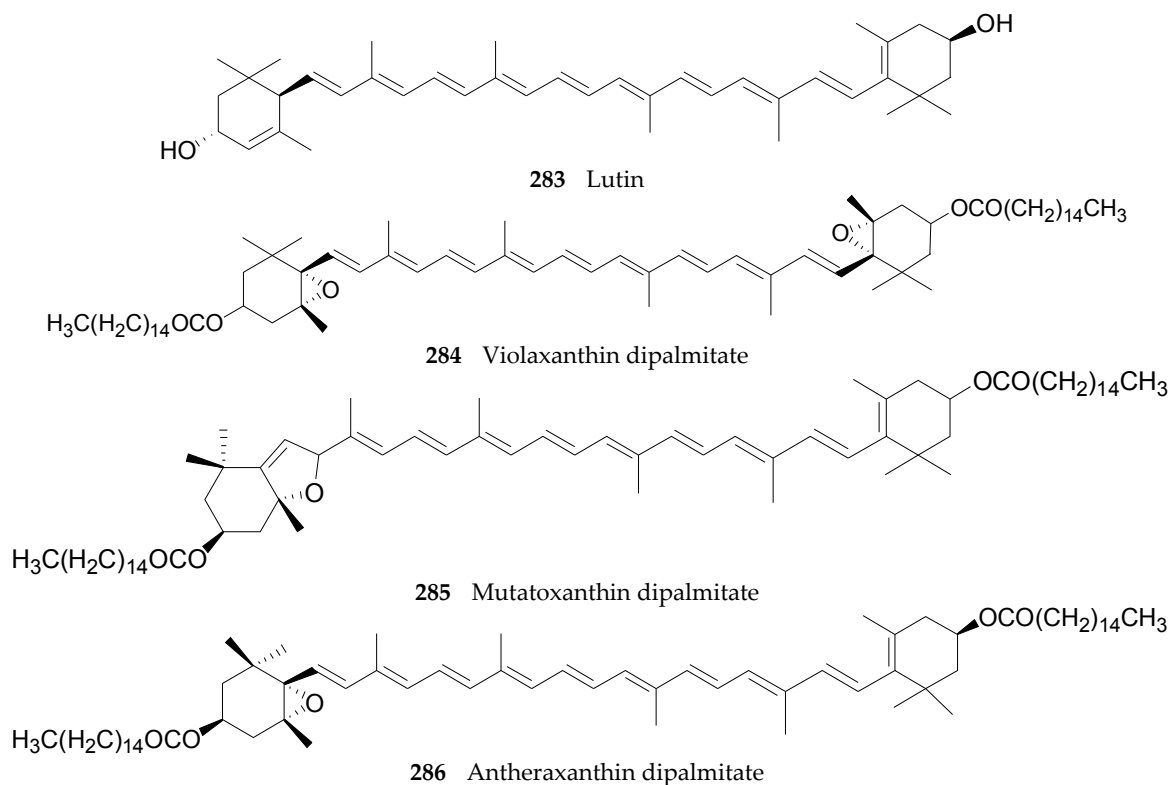


Figure 20. Chemical structures of compounds 271 and 274.

Table 12. Chemical structures of compounds 275–282.

275–282				
No.	Compounds	R ₁	R ₂	Source
275	β-Carotene	H	H	<i>L. barbarum</i>
276	β-Cryptoxanthin	OH	H	<i>L. barbarum</i>
277	Zeaxanthin	OH	OH	<i>L. barbarum</i>
278	Zeaxanthin monopalmitate	OCO(CH ₂) ₁₄ CH ₃	OH	<i>L. barbarum</i>
279	Zeaxanthin dipalmitate	OCO(CH ₂) ₁₄ CH ₃	OCO(CH ₂) ₁₄ CH ₃	<i>L. barbarum</i>
280	Zeaxanthin monomyristate	OH	OCO(CH ₂) ₁₂ CH ₃	<i>L. barbarum</i>
281	Zeaxanthin dimyristate	OCO(CH ₂) ₁₂ CH ₃	OCO(CH ₂) ₁₂ CH ₃	<i>L. barbarum</i>
282	β-Cryptoxanthin palmitate	OCO(CH ₂) ₁₄ CH ₃	H	<i>L. barbarum</i>



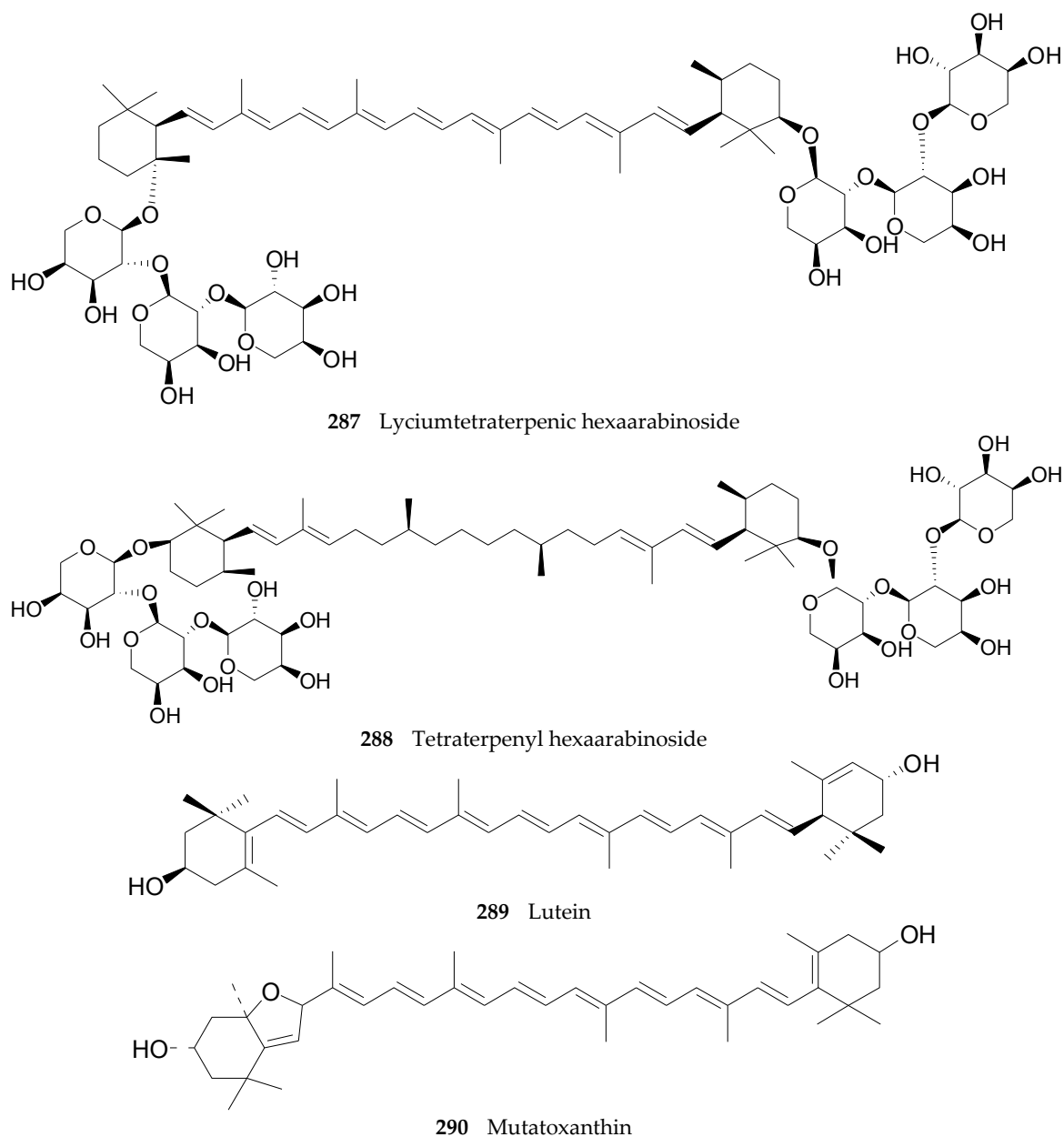
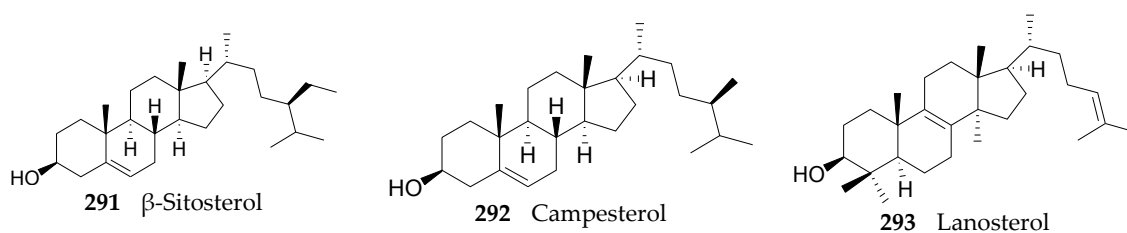
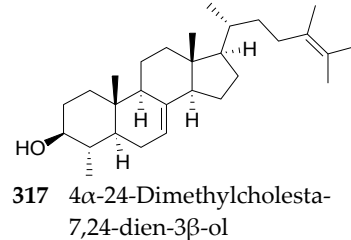
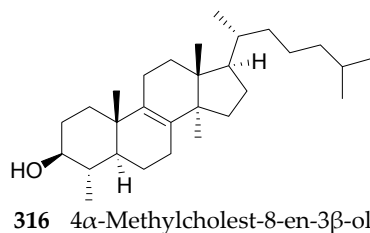
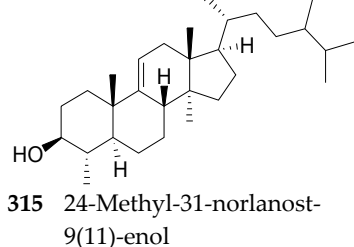
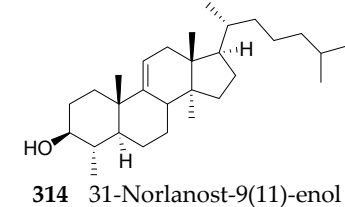
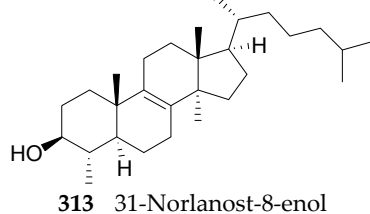
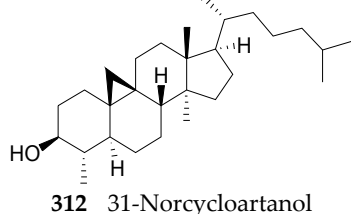
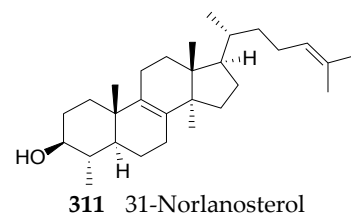
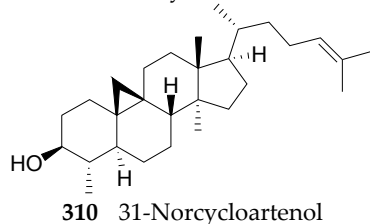
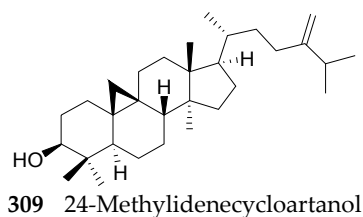
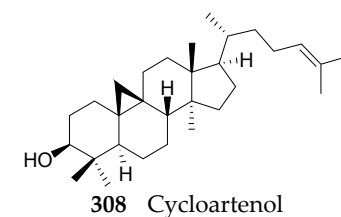
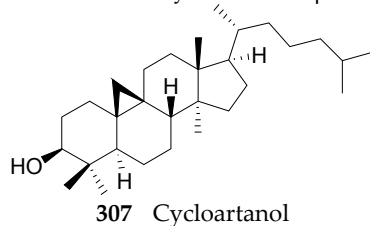
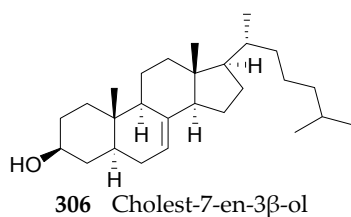
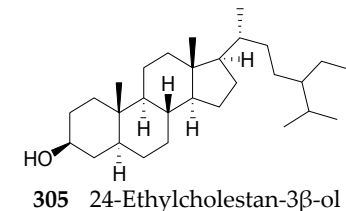
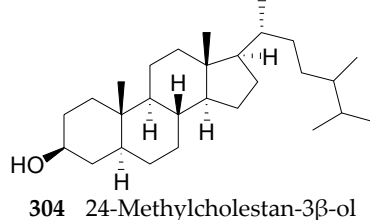
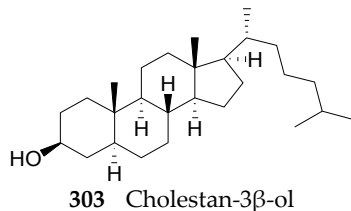
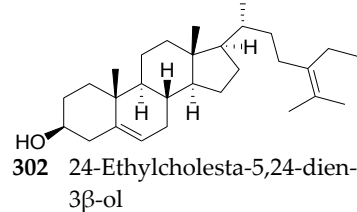
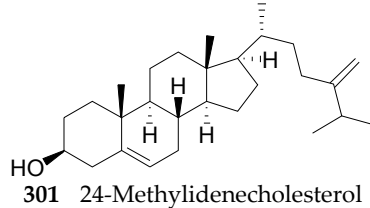
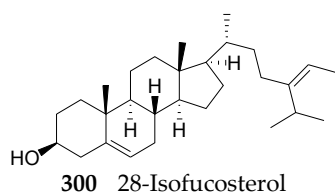
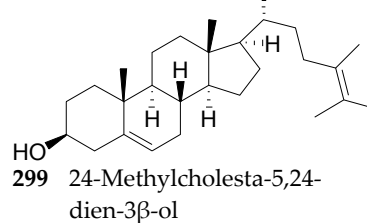
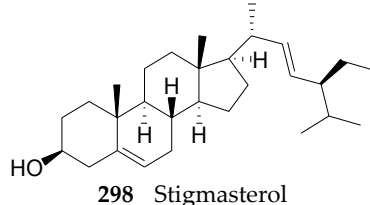
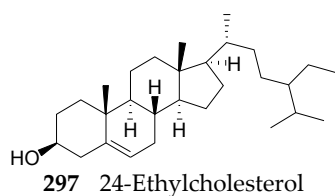
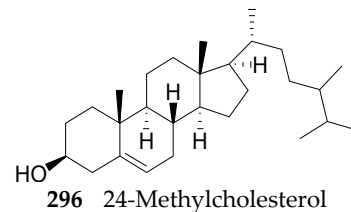
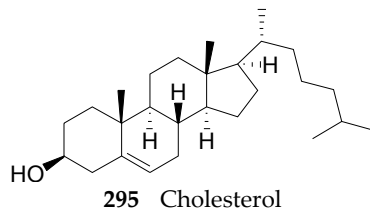
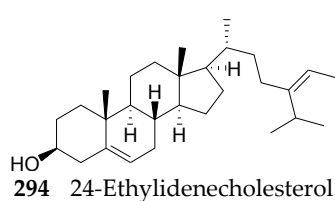


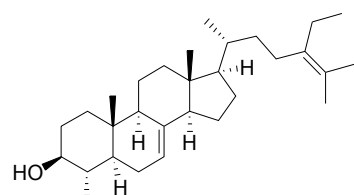
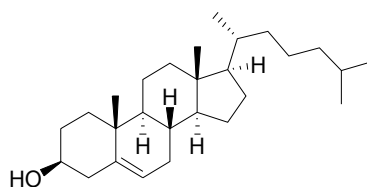
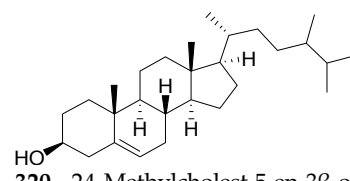
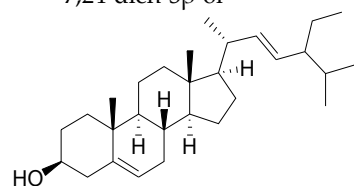
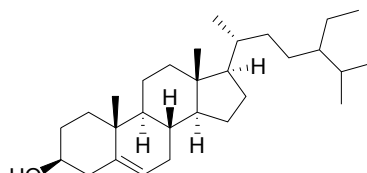
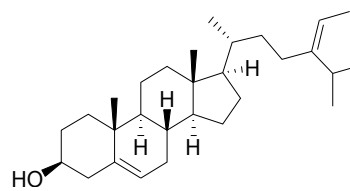
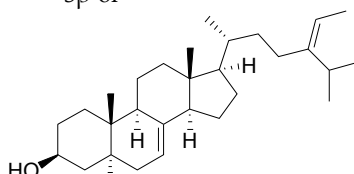
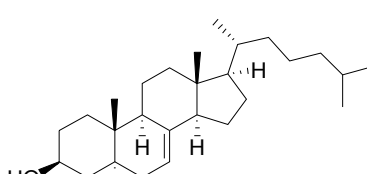
Figure 21. Chemical structures of compounds 283–290.

2.2.11. Sterols, Steroids, and Their Derivatives 291–347

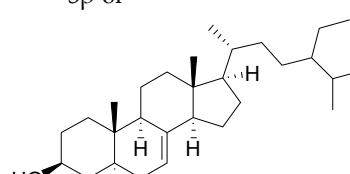
Fifty-seven sterols, steroids, and their derivatives **291–347**, listed in Figure 22, have been identified from the genus *Lycium*, mainly from the seeds and the fruits. Compounds **293** and **343** were identified from the flowers of *L. barbarum* [130], **291–292**; **295**, **298**, **319–324** and **337–339** were identified from the fruits of *L. chinense* [23,35,52,63,107,131]; **341** **342**, **346** and **347** were identified from the leaves of *L. chinense* [132,133]; **336** and **340** were identified from the root bark of *L. chinense* [80,121]; **294** was identified from the seed of *L. ciliatum* [66]; all others were identified from the seed of *L. chinense* [134–137] **344** and **345** were identified from the seeds of *L. barbarum* [138].



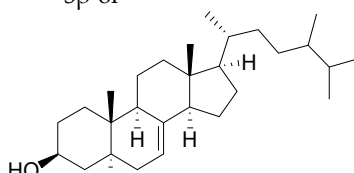


318 24-Ethyl-4 α -methylcholesta-7,24-dien-3 β -ol319 Cholest-5-en-3 β -ol320 24-Methylcholest-5-en-3 β -ol321 24-Ethylcholesta-5,22-dien-3 β -ol322 24-Ethylcholest-5-en-3 β -ol323 24-Ethylidenecholest-5-en-3 β -ol324 24-Ethylidenecholest-7-en-3 β -ol

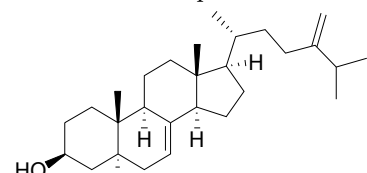
325 Lophenol



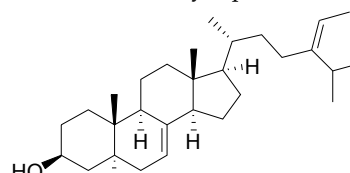
326 24-Ethyllophenol



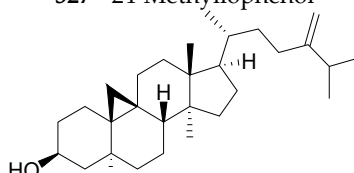
327 24-Methyllophenol



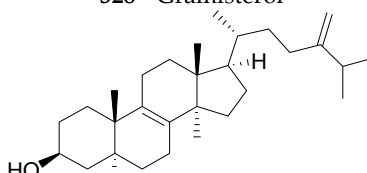
328 Gramisterol



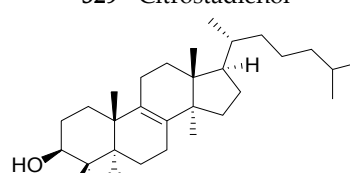
329 Citrostadienol



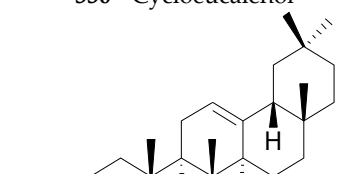
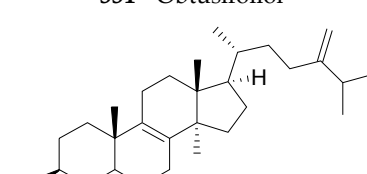
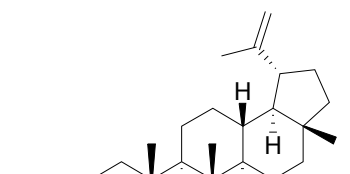
330 Cycloeucalenol



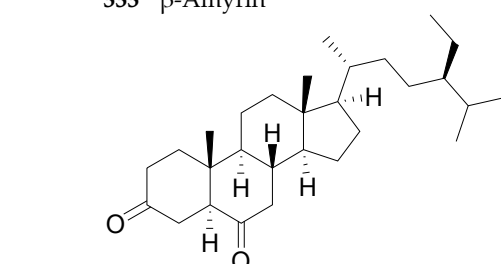
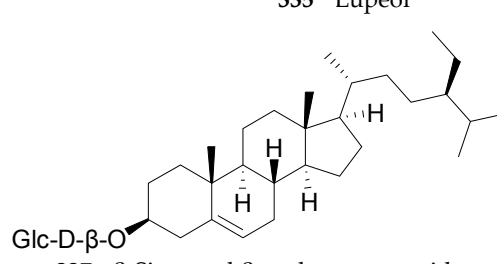
331 Obtusifoliol



332 Lanost-8-enol

333 β -Amyrin334 24-Methylenelanost-8-enol(24-methylenelanost-8-en-3 β -ol)

335 Lupeol

336 5 α -Stigmastane-3,6-dione337 β -Sitosterol β -D-glucopyranoside

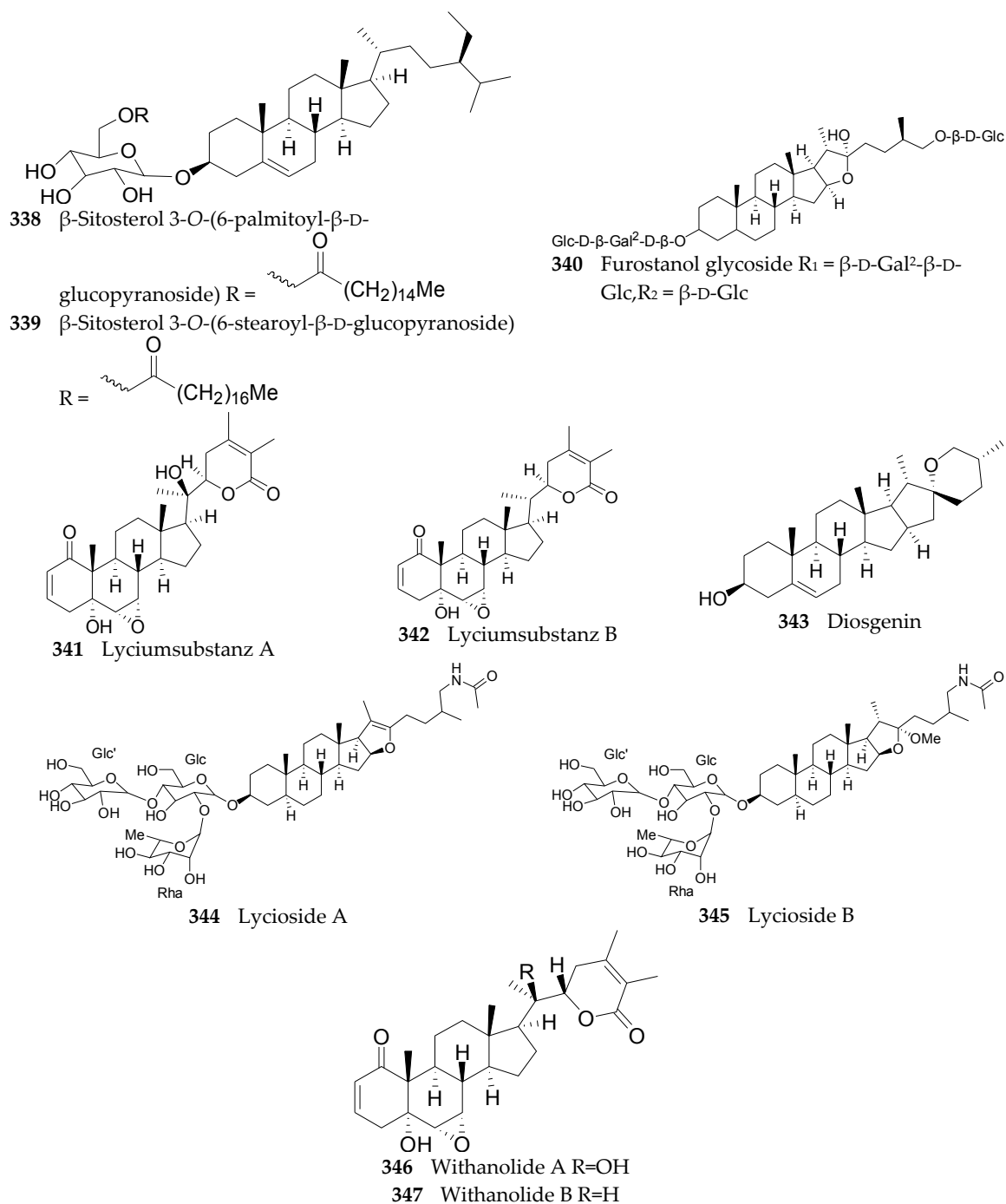


Figure 22. Chemical structures of compounds 291–347.

2.2.12. Peptides 348–352

Five peptides have been isolated from the root bark of *L. chinense* [80,139], including one dipeptide, lyciumamide (**348**), and four octapeptides, called lyciumins A–D (compounds **349–350**), illustrated in Figure 23.

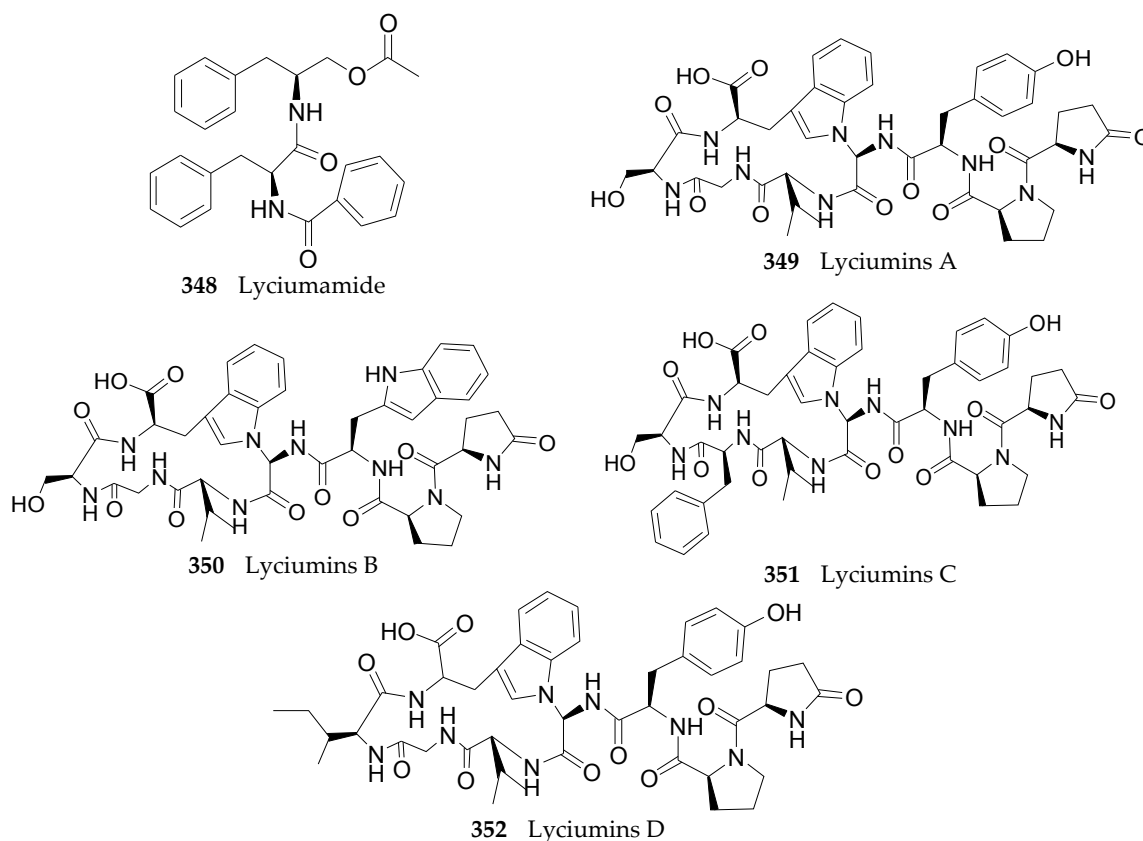


Figure 23. Chemical structures of compounds 348–352.

2.2.13. Other Compounds 353–355

Other than what has already been mentioned, a few other chemical constituents, listed in Figure 24, were also isolated from the genus *Lycium*. Digupigan A (353), 2-*O*-(β -D-glucopyranosyl)ascorbic acid (354) and *p*-hydroxybenzaldehyde (355) also have been obtained from the root bark of *L. chinense*, the fruits of *L. chinense*, and the fruits of *L. barbarum* [75,76,121,137,140,141], respectively. Many minerals, amino acids, and proteins have also been found in the genus *Lycium*, such as Ca, Mg, Zn, Fe, aminoethanesulfonic acid, γ -aminobutyric acid (GABA), Mn-SOD, etc. [121,142,143].

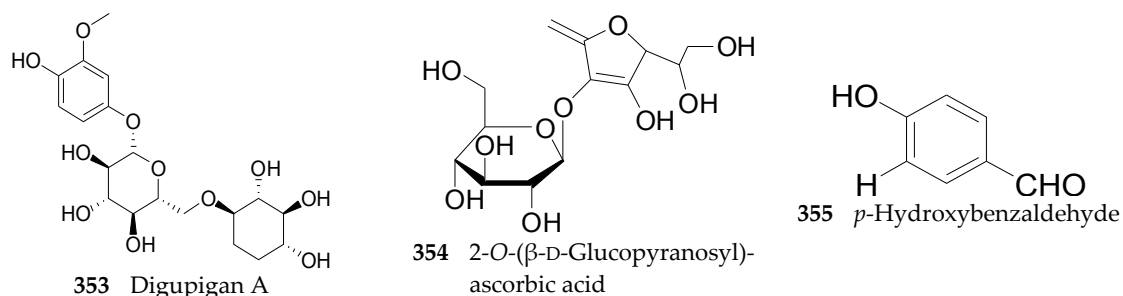


Figure 24. Chemical structures of compounds 353–355.

3. Discussion

Lycium species are of valuable medicinal, nutritional and functional significance, and have been studied in terms of their chemical compounds. Phytochemical investigations on eight different species, have resulted in the isolation of at least 355 constituents up to July of 2016. Research on chemical compounds has concentrated mainly on *L. barbarum* and *L. chinense*. Therefore, future phytochemistry research should be focused on the other species in *Lycium* genus. In addition, diverse plant parts (i.e., the flowers, leaves, seeds) have also been testified to contain new constituents, most of which possess the novel chemical structures. Polysaccharides play a particularly significant role in

exerting pharmacological actions. A specific class of polysaccharides, abbreviated as LBP, is used as biomarker in the 2015 Chinese Pharmacopoeia as a measure by which wolfberry is qualified. At present, LBP in products or in pharmacological studies usually are polysaccharide mixtures with heterogeneity and polydispersity. On the other hand, development of new separation, detection techniques will greatly benefit the phytochemical isolation and structural elucidation of LBP. There is a growing recognition that not only the LBP, but also the plant secondary metabolites may have the potential active ingredients, while most of the research on goji berry was LBP rather than small molecule substances, so more intensive studies of goji berry are required to shed some light on these compounds.

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Author Contributions: Dan Qian and Luqi Huang conceived and designed the paper; Dan Qian wrote the paper and Luqi Huang reviewed the paper. Yaxing Zhao and Guang Yang discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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