

Research progress of active compounds and pharmacological effects in *Akebia trifoliata* (Thunb) koidz stems

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Abstract. *Akebia trifoliata* (Thunb) koidz contains a variety of active ingredients, exerting the promising pharmacological actions. Its stems and fruits are legally recorded as herbal medicines in the Chinese Pharmacopoeia. However, different parts of *Akebia trifoliata* stems have different effects. In this study, the literature in the last decade was retrieved to summarize the pure compounds and pharmacological effects of *Akebia trifoliata* stems. It showed that *Akebia trifoliata* stems mainly contain a variety of oleanolic acids, ivy saponin-type triterpenoid saponins, which have antitumor, anti-inflammatory, diuretic and antibacterial actions.

1. Introduction

Formerly known as Tongcao, Mutong was first published in “ShenNong’s Classic” of Materia Medica, which has been widely used in China and other Asian countries for nearly two thousand years [1]. *Akebia trifoliata* (Thunb) koidz, as a genus *Akebia* plant of Lardizabalaceae, is one of the basic plants of the Chinese Pharmacopoeia in the 2015 edition of the Chinese Pharmacopoeia. *Akebia trifoliata* contains various active ingredients and has good pharmacology activities. The dried *Akebia trifoliata* stem has the effects of promoting diuresis and relieving stranguria, clearing away the heart-fire, and promoting lactation, and is used to treat stranguria, edema, dysuria, reddish urine, tongue sore, amenorrhea and lactation, and damp-heat [2], etc. However, the literature reports of the last decade mainly focus on anti-tumor, antidepressant, anti-inflammatory and antimicrobial effects. This article focuses on the chemical compositions and pharmacological effects of the stem of *Akebia trifoliata* in order to better study and use it.

2. Natural organic compounds in *Akebia trifoliata* stems

The stem of *Akebia trifoliata* contains various kinds of Akeboside and its aglycon, such as Hederagenin (C₃₀H₄₈O₄) or Oleanolic acid (C₃₀H₄₈O₃). The stem [3-4] also contains phenylpropanoid chemical components, phenol alcohol and phenolic glycoside chemical components, caffeoyl quinic acid chemical components, and lignan chemical components. At present, pharmacologists have



isolated 40 chemical constituents [3-7] (Table 1) from the cane of *Akebia trifoliata*, including lignans, phenolic acids and phenol glycosides, and various triterpenoids which accounts for the vast majority .

Table 1. Natural Organic compounds in *Akebia trifoliata* stems

No.	Component	Extractant	Category
1	Oleanolic-acid-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside	n-BuOH	triterpene saponins
2	Oleanolic-acid-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl	n-BuOH	triterpene saponins
3	Hederagenin-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl	n-BuOH	triterpene saponins
4	O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl-Oleanolic-acid-28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl	n-BuOH	triterpene saponins
5	3-O- β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-arabinopyranosyl-Hederagenin-28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl	n-BuOH	triterpene saponins
6	3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl-Hederagenin-28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl	n-BuOH	triterpene saponins
7	3-O- β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl-Oleanolic-acid-28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl	n-BuOH	triterpene saponins
8	3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-arabinopyranosyl-Oleanolic-acid-28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl	n-BuOH	triterpene saponins
9	3-O- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl-(1-2)- α -L-arabinopyranosyl-Hederagenin-28-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl	n-BuOH	triterpene saponins
10	2 α ,23-dihydroxy-3 β -sulfoxy lean-12-en-28-oic acid-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl	n-BuOH	triterpene saponins
11	2 α ,23-dihydroxy-3 β -sulfoxy lean-12-en-28-oic acid-O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl	n-BuOH	triterpene saponins
12	3 β -[(O- β -D-glucuronopyranosyl-(1 \rightarrow 3)-O-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- α -L-arabinopyranosyl)oxy] olean-12-en-28-oic acid	n-BuOH	triterpene saponins
13	(4-hydroxy-3,5-dimethoxybenzene methanol)	water	phenol
14	[erythro-1-phenyl-(4'-hydroxy-3'-methoxy)-2-phenyl-(4"-hydroxy-3"-methoxy)-1,3-propanediol]	water	phenol
15	[threo-1-phenyl-(4'-hydroxy-3'-methoxy)-2-phenyl-(4"-hydroxy-3"-methoxy)-1,3-propanediol]	water	phenol
16	[(7S,8S)-1-(4-hydroxy-3,5-dimethoxyphenyl)-1,2,3-propanetriol]	water	phenol
17	[2-(4-hydroxy-3-methoxyphenyl)-ethanol-1-O- β -D-glucopyranoside]	water	phenol
18	[(7S,8S)-1-(4-hydroxy-3,5-dimethoxyphenyl)-1,2,3-propanetriol-2-O- β -D-glucopyranoside]	water	phenol
19	neochlorogenic acid methyl	EtOAc	caffeoylquinic acid
20	cryptochlorogenic acid methylester	EtOAc	caffeoylquinic acid
21	chlorogenic acid methyl	EtOAc	caffeoylquinic acid
22	methyl 3, 5-di-O-caffeoyl quinate	EtOAc	caffeoylquinic acid
23	methyl 3, 4-di-O-caffeoyl quinate	EtOAc	caffeoylquinic acid
24	methyl 4, 5-di-O-caffeoyl quinate	EtOAc	caffeoylquinic acid
25	Calceolarioside B	EtOAc	phenylpropanoid

26	Osmanthuside E	EtOAc	phenylpropanoid
27	dunalianoside C	EtOAc	phenylpropanoid
28	dunalianoside D	EtOAc	phenylpropanoid
29	2-(4-hydroxyphenyl)ethyl-(6-O-feruloyl)- β -D-		
30	Glucopyranoside	EtOAc	phenylpropanoid
31	Aesculetin	EtOAc	phenylpropanoid
32	caffeic acid	EtOAc	phenylpropanoid
33	Oleanolic acid	EtOAc	triterpene saponins
34	6,7,10-trihydroxy-8-octadecenoic acid	EtOAc	Organic acid
35	tetratriacontane	EtOAc	Other
36	2,6-dimethoxy-1,4-benzoquinone	EtOAc	Other
37	β -sitosterol	EtOAc	steroid
38	Vanillic acid	EtOAc	Organic acid
39	Aketrilignoside A	water	lignan glycosides
40	Aketrilignoside B	water	lignan glycosides

3. Pharmacological effects

3.1. Anti-tumor effect

The oleanolic acid aglycone (or ivy sapogenin) and its derivatives [8-14], the main constituent of *Akebia trifoliata*, have liver protection and anti-tumor effects. Among them, the anti-tumor mechanism of oleanolic acid mainly regulates signaling pathway, arrests cell cycle, induces apoptosis, inhibits tumor angiogenesis, and resists invasion [15]. In addition, some researchers studied the inhibitory effects of ivy aglycone on colon cancer, gastric cancer, head and neck cancer, prostate cancer, breast cancer, and lung cancer [16-20]; its anti-tumor mechanisms regulate signal pathway, promote apoptosis, and resist invasion and migration. *Aesculetin* isolated from the ethanol extract of stems has inhibitory effects on human hepatoma cells HepG2, A549 lung cancer cells, human T lymphoblastic leukemia cells and human gastric cancer cells *in vitro* [21]. The possible mechanism of aescin induces apoptosis of tumor cells: promotes the release of cytochrome C from mitochondria, activates Caspase-9 to induce apoptosis, activates caspase-3 as a substrate of Caspase-9 to induce apoptosis, and regulates Bcl-2 /Bax gene expression causes programmed cell death; ADP-ribose polymerase (ADP-ribose) inactivates shear-induced cell apoptosis [22] (Figure 1).

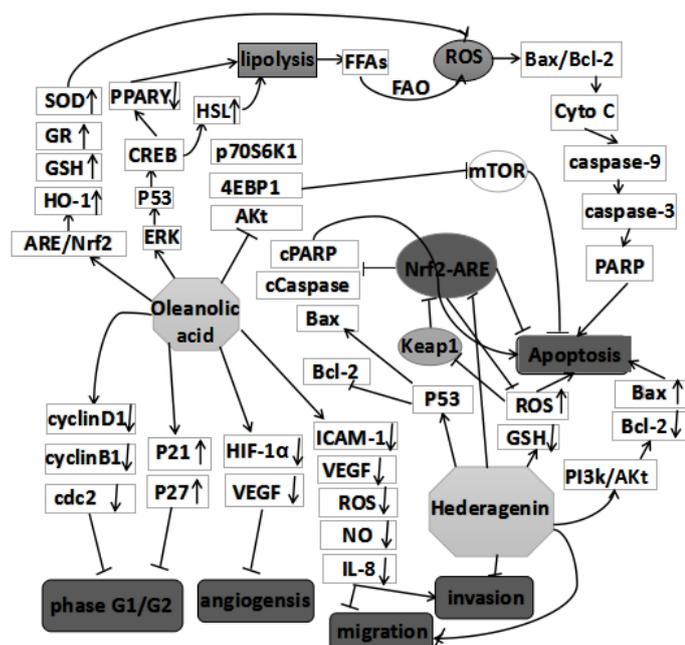


Figure 1. The signaling pathway of inhibiting tumor cells of the compounds of *Akebia trifoliata* stem.

3.2. Antidepressant effect

A preliminary study was conducted on the antidepressant activity of ivy glycosides using a corticosterone-induced PC12 cell injury model and a classic behavioral despair animal model (ie, mouse suspension and forced swimming) to detect hippocampal SOD. Activity and malondialdehyde (MDA) content, the results of the ivy saponin can improve the survival rate of PC12 cells damaged by corticosterone. The mouse experimental results showed that after an acute single dose, compared with the control group, the low dose (10 mg/kg) of ivy saponin significantly shortened the mouse tail suspension time. After 2 weeks of continuous gavage administration, ivy saponin reduced the immobility time of the mice without affecting the ability of the mice to move autonomously in the tail-suspended and forced-swimming experiments compared to the control group. Saponin has a certain anti-depressant effect, ivy aglycone can increase SOD activity in the hippocampus, and significantly reduce the MDA content. Its mechanism may be related to regulating the body's oxidative - antioxidative enzyme activity, reduce the peroxidation damage [23].

3.3. Anti-inflammatory effect

Inhibition of inducible nitric oxide synthase (iNOS) by phenolic alcohols and phenolic glycoside compounds was performed by measuring lipopolysaccharide (LPS)-induced NO production in RAW264.7 macrophage. Among them, nitric oxide is a strong signal molecule that reacts with superoxide anion to form peroxynitrite anion, which is a strong oxidant and can cause lipid peroxidation, protein tyrosine nitration, thiol oxidation Etc., mediate the occurrence of many diseases. As a result, it was found that phenolic alcohols and phenolic glycoside compounds (1-6) of *Akebia trifoliata* could inhibit the production of NO by RAW264.7 cells induced by LPS and inhibit the occurrence of inflammation [24].

3.4. Antimicrobial effect

The aqueous solution of the *Akebia trifoliata* was used as a continuous volumetric dilution of the nutrient broth in the test tube (the total volume per test tube was 1 mL), and the final concentration of the drug for inhibiting the bacteria was 200, 100, 50, 25, and 12.5 mg/mL. Add a certain amount of test bacteria dilution (10 μ L of bacteria solution per test tube) to each test tube, and incubate at 37°C for 18 h. Compare with the negative control tube, then transfer to a solid medium to observe the presence or absence of bacterial growth, determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The results showed that the *A. trifoliata* water extract had significant effects on *Streptococcus pneumoniae* and *Shigella*, and had a certain antibacterial effect on *Escherichia coli* and *Staphylococcus aureus* [25].

4. Summary

The literature of the chemical constituents and pharmacological effects of *Akebia trifoliata* stems in the last decade indicates that the main active ingredients (see Figure 2 to Figure 6) found include triterpenoids, phenols and phenol glycosides, caffeoyl quinic acids, phenylpropanoids, and lignans, a total of 40 species. There are four main types of triterpenoid compounds, namely oleanane, oligostanane, lubutol, and wusuan. While the site modification and modification of oleanolic acid is mainly focused on 3-hydroxy and 28-carboxyl groups, wherein the esterification or amidation of 3-hydroxy glycosylation and the 28-carboxyl can effectively improve the anti-tumor activity of oleanolic acid [26]. Phenylpropanoid glycosides are usually based on β -D-glucopyranose as the central sugar nucleus, substituted phenylethyl as its aglycone, and substituted cinnamoyl as ester bond with sugar core. Additional sugars can also be linked to the C6 and C3 positions of the central sugar nucleus, affecting antitumor activity through structural modifications [11]. The pharmacological effects of the main active ingredients of *Akebia trifoliata* include anti-tumor, anti-inflammatory, anti-depression, and diuretic effects. However, some caffeoylquinic acids have not been found to have pharmacological effects at present, but their derivatives and analogues have been found to have

antioxidative activity, anti-inflammatory activity, and anti-microbial effect, etc., pending further researches [27].

In summary, the stem of *Akebia trifoliata* has high utilization rate and medicinal value, and has broad prospects for the development and application of medicine, and is worthy of in-depth study.

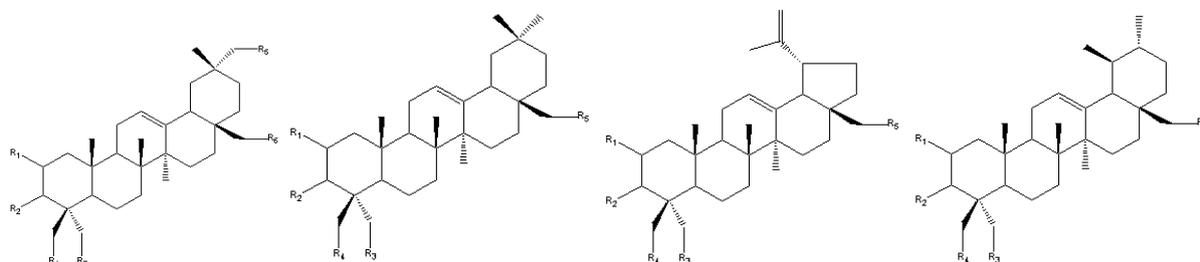


Figure 2. Polycyclic hydrocarbons' general formulas.

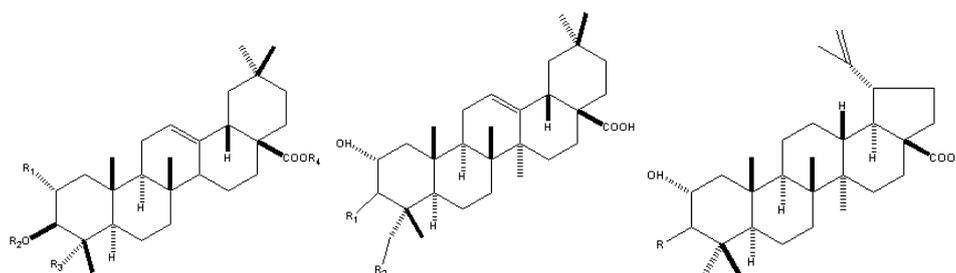


Figure 3. Triterpene saponins' general formulas.

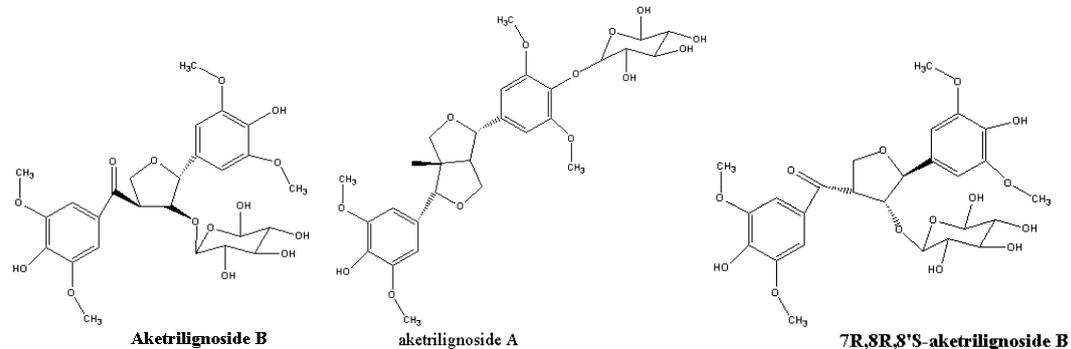
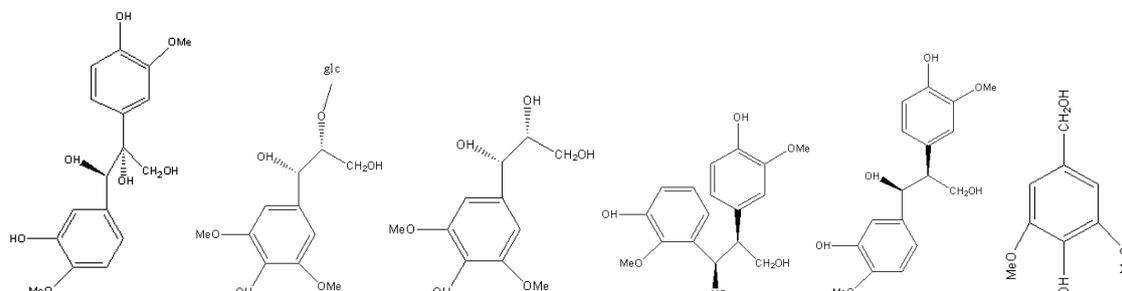


Figure 4. Lignans' structural formulas.



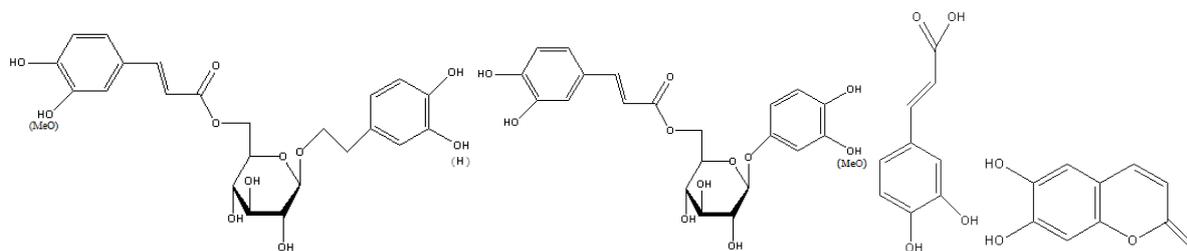


Figure 5. Phenols' and Phenylpropanoids' structural formulas.

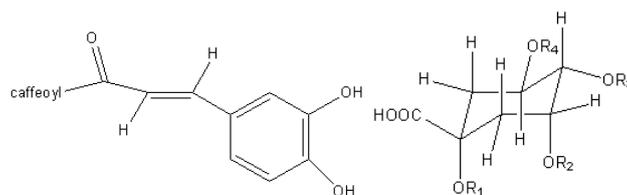


Figure 6. Caffeoyl quinic acids' general formulas.

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