Review

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Chemical constituents and bioactivities of *Colla corii asini*

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Summary **In China, *Colla corii asini* is a health-care food and traditional Chinese medicine widely used in life-nourishing and clinical hematic antanemic therapy for more than 2,000 years. In this paper we compiled the chemical constituents isolated and detected from *Colla corii asini* including amino acids, proteins/gelatins, polysaccharides, volatile substances, inorganic substances, *etc*. Meanwhile we investigated the biological activities of *Colla corii asini*, which have been reported over the past few decades, including, hematologic diseases inhibitory activities, anti-aging activity, antitumor activity, immunomodulatory activity, bone repair activity, anti-inflammatory activity, antifatigue activity, *etc*. However, few reports on the relationships between the chemical constituents and bioactivities have been found, further studies of *Colla corii asini* are still necessary to facilitate research and development in the future.**

Keywords: *Colla corii asini*, chemical constituents, biological activities

1. Introduction

Equus asinus Linnaeus (*Equus asinus* L, Figure 1A), commonly known as domesticated ass or donkey, is widely distributed in the northeast, north and northwest of China. The skin of *Equus asinus* L. has long been used as the key raw material to prepare *Colla corii asini* (*E'jiao, A'jiao*). *Colla corii asini* (Figure 1B), a gelatin-like block shaped preparation, belongs to the minority of top-grade traditional Chinese medicine (TCM) which should be obtained through a refining process after water extraction from *Equus asinus* L. skin (*1*). In China, *Colla corii asini* is a health-care food and TCM widely used in life nourishing and clinical hematic antanemic therapy for more than 2,000 years (*2,3*). In 2013, the sales of *Colla corii asini* have reached nearly 2 billion *Yuan*.

Studying active compounds is important for the development of TCM. These compounds could be meaningful for the understanding of mechanisms of action, and could constitute a promising bio-resource for the development of potential drugs and value-added products. Although little study to date has addressed the pharmacological action of the chemical compounds from *Colla corii asini*, the chemical and pharmacological properties of *Colla corii asini* have been investigated (especially in China) since the 1980s (*4,5*). Several bioactive natural products, mainly gelatins and amino acids, have been reported in *Colla corii asini*.

In this review, we compiled the chemical constituents isolated from *Colla corii asini* over the past few decades. The biological activities of *Colla corii asini* and its constituents are also discussed.

2. Chemical constituents

Several classes of compounds have been isolated from *Colla corii asini*, including amino acids, proteins/gelatins, polysaccharides, volatile substances, inorganic substances, *etc*. Some of their names, **1-58**, are collected in Table 1, and some of their structures, **1-24**, are shown in Figure 2.

2.1. *Amino acids*

Amino acids are the most abundant components of *Colla corii asini*. From the 1980s, the amino acid

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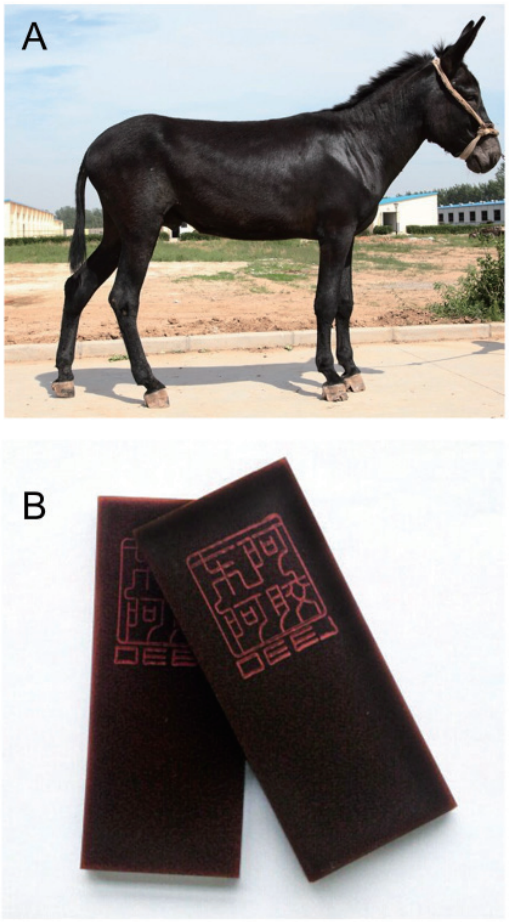

Figure 1. ***Equus asinus*** **Linnaeus (A) and** ***Colla corii asini*** **(B)**

composition in *Colla corii asini* has been determined repeatedly using automatic amino acid analyzers (*1,4,6-8*). It has been reported that 18 types of amino acids were detected in hydrolyzed *Colla corii asini*, and the total content was from 51.94% to 82.03%. Their names and respective content are collected in Table 2.

In addition, Cheng *et al*. determined 4 amino acids using a pre-column derivatization high performance liquid chromatography (HPLC) method. Besides glycine, alanine and proline that have been listed, the content of hydroxyproline was determined to be 8.99%-11.23% (*9*).

2.2. *Proteins/gelatins*

The total protein content of *Colla corii asini* was determined to be 74.56% to 84.94% using the Kjeldahl nitrogen determination method (*4*). From the refined preparation of the skin of *Equus asinus* L., the constituent proteins of *Equus asinus* L. skin could support a clue to expose the constituents of *Colla corii asini*. In 2006, three majority proteins, collagen α1 (I), collagen α2 (I) and donkey serum albumin were determined from the skin of *Equus asinus* L. Their respective content was about 12.6%, 11.67% and 19.6% in the total proteins (*10*). Then, a citric-soluble collagen and a pepsin-soluble collagen were successfully extracted from *Equus asinus* L. skin, and both were identified as type I collagen, containing two different α chains (α1 and α2) (*11*).

Gelatin is commonly considered to be the most abundant and biologically active component of *Colla*

Table 1. Chemical constituents from ***Colla corii asini***

	Name	*Ref.*
1	Dermatan sulfate	(*16,17*)
2	Methane, isothiocyanato-	(*21*)
3	9,12-Octadecadienoic acid(Z,Z)-,methyl ester	(*21*)
4	13-Octadecenal,(Z)-	(*21*)
5	Z-5-Methyl-6-heneicosen-11-one	(*21*)
6	Cyclododecanone,2-methylene-	(*21*)
7	Tetradecane,1-chloro-	(*21*)
8	Tetratriacontane	(*21*)
9	Heneicosane	(*21*)
10	Tricosane	(*21*)
11	Tetracosane	(*21*)
12	Docosane, 1-bromo-	(*21*)
13	Octadecane, 1-chloro-	(*21*)
14	7-Oxabicyclo(4,1,0)heptane,1-methyl-4-(2-methyloxiranyl)-	(*21*)
15	Oxacycloheptadecan-2-one	(*21*)
16	Naphthalene, 2-methyi-	(*21*)
17	1,1'-biphenyl,3-(1-methylethyl)-	(*21*)
18	2-Amino-6,7-dimethyl-5,6,7,8-tetrahydro-4-pteridinol	(*21*)
19	Cyclohexene,4-(4-ethylcyclohexyl)-1-pentyl-	(*21*)
20	Aristolene epoxide	(*21*)
21	p-Menth-8(10)-en-9-ol,cis-	(*21*)
22	13-Octadecenal,(Z)-	(*21*)
23	2-Dodecen-1-yl(-)succinic anhydrid	(*21*)
24	8-Hexadecenal,14-methyl-,(Z)-	(*21*)
25	Iron sesquioxide	(*6*)
26	Calcium oxide	(*6*)
27	Magnesium oxide	(*6*)
28	Potassium oxide	(*6*)
29	Sodium oxide	(*6*)
30	Titanium dioxide	(*6*)
31	Manganese dioxide	(*6*)
32	Phosphorus pentoxide	(*6*)
33	Potassium	(*4*)
34	Sodium	(*4*)
35	Calcium	(*4,6*)
36	Magnesium	(*4*)
37	Iron	(*4*)
38	Copper	(*4,6*)
39	Aluminum	(*4*)
40	Manganese	(*4*)
41	Zinc	(*4*)
42	Chromium	(*4,6*)
43	Platinum	(*4*)
44	Stannum	(*4*)
45	Plumbum	(*4*)
46	Silver	(*4*)
47	Bromine	(*4*)
48	Molybdenum	(*4*)
49	Strontium	(*4*)
50	Barium	(*6*)
51	Cadmium	(*6*)
52	Cobalt	(*6*)
53	Niobium	(*6*)
54	Nickel	(*6*)
55	Strontium	(*6*)
56	Vanadium	(*6*)
57	Lanthanum	(*6*)
58	Thorium	(*6*)

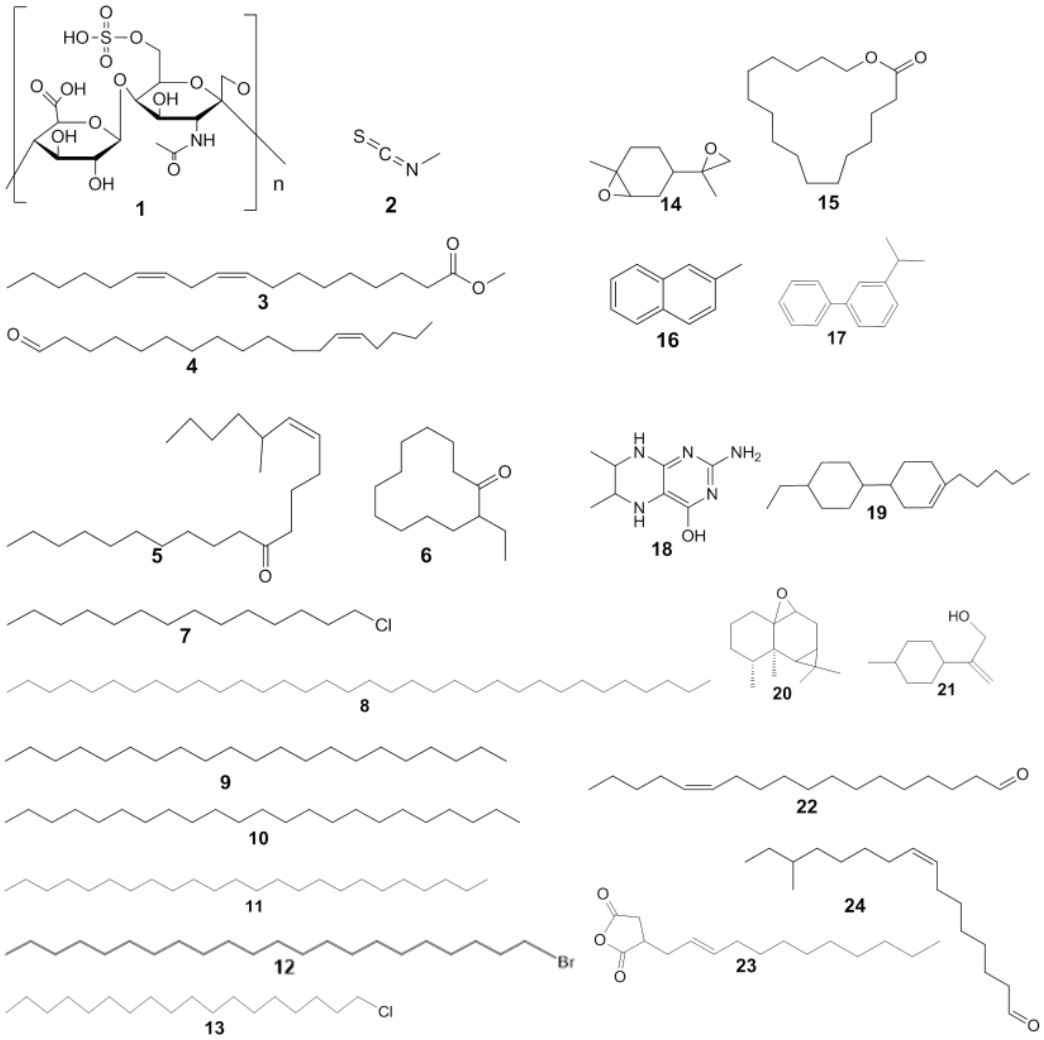

Figure 2. Chemical structures of compounds 1-24.

corii asini (*1-3*). It is a mixture of peptides and proteins produced by partial hydrolysis of collagen (*12-14*). The content of hydroxyproline which marked the composition of gelatin was determined to be 8.99-11.23% in *Colla corii asini* (*9*). In 2012, in order to distinguish *Colla corii asini* from other animal skin glue, the marker for *Colla corii asini* gelatin was identified as the fragment GEAGPAGPAGPIGPVGAR by an ultra-performance liquid chromatography/ time- of-flight mass spectrometry (UPLC/Q-TOF-MS) sample profiling method coupled with principal component analysis (PCA) (*15*).

2.3. *Polysaccharides*

Polysaccharides are important components of the skin of *Equus asinus* L. However, so far, only dermatan sulfate (DS), **1**, was isolated from *Colla corii asini* (*16,17*). DS is a glycosaminoglycan (GAG) that is distinguished from chondroitin sulfate (CS) by the

Table 2. Contents of amino acids in *Colla corii asini*

Amino acids	Content (%)	*Ref.*
Aspartic Acid	3.37-5.14	(*1,4,6,7*)
Threonine	1.11-1.31	(*1,4,6,7*)
Serine	1.25 - 2.86	(*1,4,6,7*)
Glutamic Acid	6.27-9.01	(*1,4,6,7*)
Glycine	13.36-23.63	(*1,4,6,7,9*)
Alanine	5.33-9.22	(*1,4,6,7,9*)
Valine	1.71-2.31	(*1,4,6,7*)
Methionine	0.29-1.56	(*1,4,6,7*)
Isoleucine	0.46-1.38	(*1,4,6,7,8*)
Leucine	0.19-3.45	(*1,4,6,7,8*)
Tyronine	0-2.27	(*1,4,6,7,8*)
Phenylalanine	1.35-2.44	(*1,4,6,7,8*)
Lysine	2.42-3.57	(*1,4,6,7*)
Cysteine	0.26-0.30	(*1,4*)
Histidine	0.53-0.88	(*1,4,6,7*)
Arginine	4.54-6.76	(*1,4,6,7*)
Proline	6.52-13.50	(*1,4,6,7,9*)
Tryptophane	0.50	(*1*)
Hydroxyproline	8.99-11.23	(*9*)
NH_3	0.28-3.27	(*1,4*)
Total	51.94-82.03	(*1,4,6,7*)

presence of iduronic acid (IdoA), the C-5 epimer of D-glucuronic acid (GlcA) (*18-20*).

2.4. *Volatile substances*

In 2010, a total of 23 volatile substances, **2-24**, have been detected from *Colla corii asini* using a gas chromatograph-mass spectrometer (GC-MS). These volatile substances mainly included esters (**2-3**), ketones (**4-6**), halogenated hydrocarbons (**7-13**), heterocyclic compounds (**14-18**) and others (**19-24**) (*21*).

2.5. *Inorganic substances*

The contents of 8 inorganic oxides (**25-32**) and 26 inorganic elements (**33-58**) of *Colla corii asini* were qualitative and quantitative determined using atomic absorption spectroscopy (AAS), emission spectra (ES), and an inductively-coupled plasma emission spectrometer (ICP-AES). Calcium oxide (0.18%) and sodium (0.35%) respectively represent the highest content of inorganic substances (*4,6*).

3. Biological activities

3.1. *Hematologic diseases inhibitory activities*

Colla corii asini was reported to treat various hematologic diseases, including anemia, aleucocytosis, thrombopenia, *etc*.

3.1.1. *Anti-anemia Activity*

As a TCM, *Colla corii asini* has been widely used in clinical hematic antanemic therapy in China for more than a thousand years (*1*). However, until recent years, little study had addressed the effect of *Colla corii asini* on the anti-anemia process using modern pharmacological methods. From 2007 to 2011, Wu and co-workers investigated the hematopoietic effect and mechanism of fractions from enzyme-digested *Colla corii asini* on anemic mice separately induced by 5-fluorouracil, γ-rays, or cyclophosphamide, *etc*. (*3,22-24*). The results suggested that fractions from the enzyme-digested *Colla corii asini* promoted hematopoiesis by activating immature granulocyte and erythroid cells, partly by stimulating granulocyte-macrophage colony stimulating factor (GM-CSF) in all mice separately induced by 5-fluorouracil, γ-rays, or cyclophosphamide (CTX), *etc*. Dissimilarly, fractions promoted hematopoiesis partly by stimulating erythropoietin (EPO) secretion and suppressing serum transforming growth factor (TGF-β) release in 5-fluorouracil induced mice, partly by stimulating interleukin-6 (IL-6) secretion and elevating the reactive oxygen species (ROS) scavenging ability in γ-ray induced mice, and partly by stimulating CD34 secretion and increasing the ratio of S-phase-cells in CTX induced mice.

In 2011, Song *et al*. identified the curative effect of *Colla corii asini* on anemic mice induced by phenylhydrazine hydrochloride (*25*). In 2012, Peng *et al*. suggested that Radix Angelica Sinensis combined with *Colla corii asini* could improve hypoferric anemia *in vivo* in rats induced by low iron feed (*26*).

3.1.2. *Thrombocytopenia therapeutic activity*

Hemostasis has been recorded to be another important activity of *Colla corii asini* for a thousand years. In modern pharmacology research theory, platelets (PLT) are considered to be a key factor in hemostasis. From 2002 to 2006, Wei and co-workers observed the clinical curative effect of *Colla corii asini* in treating peripheral thrombocytopenia patients with malignant tumors (including lung cancer, esophageal cancer, liver cancer, gastric cancer, breast cancer and lymphoma) after radiotherapy or chemotherapy. The results showed that a large dose of *Colla corii asini* could significantly increase PLT levels ($p < 0.05$) and stimulate the activity of bone marrow stem cells (particularly the megakaryocytic cells) in these radio- or chemo-therapeutic cancer patients (*27-29*).

3.1.3. *Leukocyte increasing activity*

In 2002, Zhang *et al*. reported that *Colla corii asini* could exert clinical curative effects on leukopenia patients caused by clozapine (*30*). Since then the effects and mechanisms of *Colla corii asini* to increase leukocytes gradually attracted attention from many researchers. In 2005 and 2009, studies separately performed by Zheng *et al*. and Xu *et al*. showed that *Colla corii asini* could increase leukocytes *in vivo* in CTX-induced leukopenia in mice. In the model mice, *Colla corii asini* could improve thymus index (TI) and spleen index (SI), and bone marrow cells through recovering the life cycle of bone marrow karyocyte cell, raise the contents of $CD34^+$ cells and red blood cells (RBC), and increase the level of hemoglobin (HB), interleukin-3 (IL-3) and GM-CSF (31,32). In 2011, Ying *et al*. identified that *Colla corii asini* could also improve leukopenia symptoms in rats induced by CTX (*33*).

3.2. *Anti-aging activity*

As a life-nourishing food in China, *Colla corii asini* has always been considered to have an anti-aging effect (*1*). In 2001, Li *et al*. proved that *Colla corii asini* could improve the damage to learning and memory in lead-inducied rats. In addition, the total antioxidant capacity of the hippocampus was detected to be increased significantly in *Colla corii asini* treated model rats

($p < 0.01$) (*34*). The free radical theory of aging was conceived by Harman in 1956 (*35*). Abundant evidence suggests that oxidative stress plays a central role in the process of biological aging (*36*). In 2012, the potential anti-aging effect of *Colla corii asini* and related mechanisms was systematically investigated by Wang *et al*. using D-galactose (gal) induced aged model mice. Results indicated that *Colla corii asini* might have an effect to suppress the aging process through enhancing the antioxidant activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), scavenging free radicals such as malondialdehyde (MDA), and modulating aged-related gene expression (p16, p21) (*1*).

3.3. *Antitumor activity*

In 2005, Liu and co-workers performed serial research on the antitumor activity of *Colla corii asini* using modern pharmacological experiments. Their studies revealed that *Colla corii asini* could exhibit inhibitory effects on the growth of cancer cells *in vitro*, such as leukemia K562 cells and lung cancer PG cells. The growth-inhibitory effects were associated with apoptosis regulated by p53 and telomerase expression (*37-40*). Furthermore, their study also proved that *Colla corii asini* could suppress tumor growth *in vivo* in S180 sarcoma-bearing-mice, and prolong survival time of these model mice (*41*).

3.4. *Immunomodulatory activity*

In 2005, Zheng *et al*. reported that *Colla corii asini* could improve the proliferation and activation of depressed lymphocytes taken from radio-therapy cancer patients *in vitro*, as well as raise the ratio of Th1/Th2 cells, and the proportion of T cells and NK cells (*42*). Furthermore, the studies of Zhang *et al*. and Song *et al*. showed that *Colla corii asini* could enhance nonspecific and cellular immunity *in vivo* in hypo-immune mice induced by hydrocortisone. In these *Colla corii asini* treated model mice, delayed-type hypersensitivity (DTH), carbon clearance index, and paw swelling index were improved; SI and TI were increased; transformation ability of spleen lymphocytes and phagocytosis ability of celiac macrophages were enhanced; and the level of hemolysin, IL-3, and interferon-γ (IFN-γ) were improved. All the indexes were improved obviously compared to the model mice ($p < 0.05$) (*43,44*). Furthermore, Huang *et al*. proved the positive immunomodulatory activity of *Colla corii asini in vivo* in chickens using the erythrocyte rosette test (*45*).

3.5. *Bone repair activity*

In 2004, Gao *et al*. first reported that *Colla corii asini* could improve bone repair *in vivo* in the early- and meta-phase of bone repair in tibial drilled SD rats. The improvement might be associated with the proliferation of chondrocytes and osteoblasts regulated by expression of pro-collagen mRNA type I, II, III, and TGF-β1 mRNA. On the other hand, genes related to blood vessel formation, such as bone morphogenetic protein (BMP-2mRNA) and vascular endothelial growth factor (VEGF-mRNA) were influenced little by *Colla corii asini* (*46*). In 2009, a study by Chang *et al*. showed that *Colla corii asini* had no effect on the multiplication of Wistar rats' osteoblasts, but a positive effect on the differentiation of osteoblasts through promoting synthesis of alkaline phosphatase (ALP) *in vitro* (*47*).

3.6. *Anti-inflammatory activity*

In 2006, the effect of *Colla corii asini* to inhibit airway inflammation was first reported by Zhao *et al*. in asthmatic rats. The result showed that *Colla corii asini* could regulate the ratio of Th1/Th2 by decreasing Th2, and inhibit the shift of eosinophils from peripheral blood to the lungs (*48*). The clinical curative effects of *Colla corii asini* on digestive system inflammation such as ulcerative colitis, chronic atrophic gastritis, and peptic ulcers were demonstrated by Wu *et al*. and Chen respectively (*49,50*).

3.7. *Antifatigue activity*

In 2011, *Colla corii asini* was proved to have antifatigue activity simultaneously by Song *et al*. and Li *et al*. in mice using a weight-loaded swimming test. The results showed that *Colla corii asini* could increase liver index, promote the synthesis of liver glycogen and HB, and decrease the product of blood lactic acid and blood urea nitrogen in weight-loaded swimming mice (*25,51*).

3.8. *Other activities*

In 2009, Su *et al*. explored the clinical effect of *Colla corii asini* to improve uterine receptivity in controlled ovarian stimulation. The result suggested that *Colla corii asini* could improve the blood supply of the uterus, resulting in the improvement of endometrial thickness (*52*). Besides the above mentioned activities, the clinical therapeutic effect of *Colla corii asini* used to treat postoperative incision fat liquefaction and malignant hematuria were also reported in recent years (*53,54*).

4. Discussion

Colla corii asini (*E'jiao, A'jiao*), a gelatin-like preparation obtained through stewing and concentrating material from *Equus asinus* L. has been used as traditional Chinese medicine for more than 2000

years. About 58 compounds were isolated or detected from *Colla corii asini* including mainly amino acids, proteins/gelatins, polysaccharides, volatile substances, inorganic substances, *etc*. As a health-care food and TCM, *Colla corii asini* showed a broad range of biological activities. Nevertheless, few reports on the relationships between the chemical constituents and bioactivities have been found, further studies to exploit other kinds of constituents, new biological activities and the relationships between chemical constituents and bioactivities are still necessary to facilitate further research and development.

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