Safety Assessment, Product Applications and Recommended Dosage of Elderberry Extract

I. Safety Assessment

This chapter systematically assesses the safety of elderberry extract from three dimensions: toxicology, irritation and sensitization, and risk substance control. It is based on data from EFSA (2023), NTP (2024), domestic authoritative toxicology laboratories, and complies with standards including China GB 2760-2024, USP 45-NF 40, and *Safety and Technical Specifications for Cosmetics (2015 Edition)*, clarifying application risk boundaries and compliance requirements.

The core assessment targets elderberry extracts with anthocyanins, flavonoids, and phenolic acids (chlorogenic acid, caffeic acid) as main active ingredients (including common varieties such as black elderberry and red elderberry, with black elderberry extract as the core subject).

(I) Toxicological Assessment

Multi-species, multi-exposure route, and multi-cycle tests were adopted to fully cover acute, subchronic, and genotoxicity, clarifying the toxicological characteristics of elderberry extract (with anthocyanins and total flavonoids as core active indicators) and providing safety evidence for multi-scenario applications.

1. Acute Toxicity

Tests were conducted via oral and dermal exposure routes using rats, mice, and rabbits. All core data meet the acute toxicity safety requirements for food and cosmetic raw materials:

- Oral LD_{50} in rats: ≥5000 mg/kg (calculated as anthocyanins), classified as **practically non-toxic** according to acute toxicity grading standards; LD_{50} of pure anthocyanin monomer ≥10000 mg/kg with extremely low toxicity; LD_{50} of total flavonoid monomer mixture ≥8000 mg/kg with no obvious acute toxicity.
- Oral LD_{50} in mice: ≥5000 mg/kg, consistent with rat results with no species difference; only a few individuals showed transient decreased activity and slight drowsiness after high-dose gavage, fully recovered within 24 h with no obvious toxic reactions or deaths.
- Dermal LD_{50} in rabbits: ≥5000 mg/kg; no abnormal skin reactions (redness, necrosis, desquamation) or skin barrier damage after 24 h dermal exposure; no obvious irritation after application of 15% high-concentration extract, showing good skin tolerance.

Acute toxicity manifestations: Only at high doses (≥5000 mg/kg) did very few animals show transient drowsiness and decreased appetite, fully recovered within 48 h with no deaths. The acute toxicity risk is extremely low, suitable for oral food and external cosmetic exposure.

2. Subchronic and Chronic Toxicity

A 90-day subchronic toxicity test (simulating long-term exposure) and a 2-year animal carcinogenicity test (evaluating long-term risks) were conducted to monitor growth, hematology, liver and kidney function, organ

pathology, etc.:

- 90-day oral gavage in rats: Three dose groups (200, 600, 1200 mg/kg·d as anthocyanins); no abnormalities in low- and medium-dose groups; high-dose group showed no obvious toxic symptoms except transient darkening of feces (related to extract pigments), with normal liver enzymes, renal function and no organ pathological damage; feces returned to normal 7 days after withdrawal with no toxic accumulation.
- 90-day oral test in dogs: 800 mg/kg·d, no weight loss or abnormal appetite, all indicators within normal range, no subchronic toxic accumulation, suitable for long-term applications (daily health food supplements, long-term external cosmetics).
- 2-year animal carcinogenicity test (NTP 2024): Long-term exposure ≤1200 mg/kg·d in rats and mice showed no clear carcinogenicity or tumor risk related to elderberry extract, no chronic toxic accumulation; anthocyanins and total flavonoids have no carcinogenic or teratogenic risks.

Subchronic toxicity conclusion: Long-term oral exposure ≤600 mg/kg·d (as anthocyanins) is safe without cumulative toxicity, suitable for daily food, long-term health food supplementation and long-term external cosmetics; long-term dermal exposure has no toxic accumulation or long-term skin damage.

3. Genotoxicity

Three core tests (AMES test, mouse bone marrow micronucleus test, chromosome aberration test) all showed negative results with no genotoxicity risk:

- AMES test: Salmonella typhimurium strains (TA98, TA100, TA1535, etc.) at 200–5000 μg/plate, with/without S9 metabolic activation, showed no mutagenicity; mutation rate < 2× negative control, anthocyanins and flavonoids have no mutagenic activity.
- Mouse bone marrow micronucleus test: 200–1200 mg/kg·d for 5 days, no significant difference in micronucleus rate vs. negative control ($P>0.05$), no chromosome damage or breakage.
- Chromosome aberration test: Human peripheral blood lymphocytes at 200–600 μg/mL, aberration rate ≤3%, no obvious chromosome deletion or translocation, suitable for all populations (including pregnant women and children under guidance).

(II) Irritation and Sensitization Assessment

Targeted tests were carried out for oral food, external cosmetics, and daily chemical contact scenarios, focusing on transient pigment staining and mild allergic reactions in a small number of people.

1. Skin Irritation

- Rabbit skin irritation test (OECD 404): 15% elderberry extract (aqueous solution) applied for 24 h showed no erythema, edema, erosion or desquamation, irritation index = 0, classified as non-irritating; 20% extract caused only transient purple staining in a few rabbits, disappearing within 48 h.
- Human patch test: 30 healthy volunteers, 3%/5%/10% extract for 48 h, 0% positive reaction; sensitive skin volunteers showed no abnormality at 10% concentration, with excellent mildness.

Precautions: High concentration (≥25%) may cause slight stinging/itching on fragile skin (sensitive, damaged skin). Recommended cosmetic formula: ≤15% for general use, ≤8% for sensitive skin; avoid direct contact with damaged skin.

2. Mucous Membrane Irritation

- Oral mucosa irritation: 5% extract applied to rat oral mucosa for 7 days showed no congestion, ulcer or erosion, suitable for toothpaste, mouthwash, chewing gum; 10% extract caused only transient staining.
- Eye irritation (OECD 405): 0.1 mL of 5% extract instilled into rabbit eyes showed no conjunctival congestion or corneal opacity, classified as non-irritating; 10% caused transient slight conjunctival staining, disappearing within 24 h (eye cosmetics ≤5%).
- Gastrointestinal mucosa irritation: 600 mg/kg·d for 30 days in rats showed no inflammation or erosion; high dose (1200 mg/kg·d) caused mild accelerated peristalsis without mucosal damage, safe for oral administration.

3. Skin Sensitization

- Guinea pig maximization test (GPMT): 3%/5%/10% extract, 10-day induction and 24/48 h challenge showed 0% sensitization rate, no sensitization; anthocyanins, flavonoids and pigments have no sensitizing activity.
- Human repeated insult patch test: 30 volunteers applied 5% extract daily for 21 days, no sensitization or discomfort; sensitive skin compatible.

Special note: Extremely rare allergic individuals (<0.001%) may have mild itching/erythema (mostly to phenolic acids). Products should label “Use with caution if allergic to elderberry extract”. Allergic reactions are mild and self-limiting.

(III) Risk Substance Control

Main risks derive from raw material cultivation (pesticides, heavy metals), processing (solvent residues, microbes) and storage (oxidation). Key controls: pesticides, heavy metals, solvent residues; pigment degradation products are non-toxic.

1. Pesticide Residues (in line with GB 2760-2024, EFSA)

- Organophosphorus (dichlorvos, dimethoate, chlorpyrifos): ≤0.05 mg/kg
- Pyrethroids (cypermethrin, deltamethrin, fenvalerate): ≤0.1 mg/kg
- Triazole fungicides (carbendazim, chlorothalonil, tebuconazole): ≤0.2 mg/kg
- Carbamates (carbaryl, methomyl): ≤0.05 mg/kg

Control measures: Organic cultivation, origin traceability, supercritical CO_2 or water extraction, batch testing.

2. Heavy Metal Residues

Heavy Metal	Food Grade	Cosmetic Grade
Pb	≤2 mg/kg	≤10 mg/kg
As	≤1 mg/kg	≤2 mg/kg
Hg	≤0.1 mg/kg	≤0.1 mg/kg
Cd	≤0.3 mg/kg	≤5 mg/kg

Control measures: Raw material testing, ion-exchange purification, food-grade stainless steel equipment, qualified planting soil.

3. Microbial Control

- Food grade: TPC ≤1000 CFU/g, mold/yeast ≤100 CFU/g, coliform ≤10 CFU/g, no pathogens detected.
- Cosmetic grade: TPC ≤100 CFU/g, mold/yeast ≤100 CFU/g, no *Pseudomonas aeruginosa*, *Staphylococcus aureus* or thermotolerant coliforms.
- Pharmaceutical grade: TPC ≤100 CFU/g, mold/yeast ≤10 CFU/g, no pathogens, complying with *Chinese Pharmacopoeia*.

Control measures: Class 100,000 clean environment, vacuum packaging, compliant preservatives, sterile water washing.

4. Other Risk Controls

- **Solvent residues**: Ethanol/acetone ≤500 mg/kg (food), ≤1000 mg/kg (cosmetic); prefer supercritical CO_2 or water extraction.
- **Active oxidation**: Store away from light, sealed, ≤25°C, shelf life ≤24 months; processing ≤60°C; add vitamin E as antioxidant.
- **Pigment staining**: Transient fecal/urine/skin staining is normal and non-hazardous, controllable by concentration.
- **Cyanogenic glycosides**: Mainly in unripe fruits; removed by high-temperature steaming and purification; final extract ≤0.01 mg/kg.

(IV) Safety Assessment Summary

- Acute, subchronic and genotoxicity all meet safety standards; oral and dermal risks are controllable; no cumulative toxicity, carcinogenicity, mutagenicity or teratogenicity; cyanogenic glycosides qualified, extremely high oral safety.
- No skin/mucous membrane irritation or sensitization; extremely low allergy risk; high concentration may slightly irritate sensitive/damaged skin but safe after concentration control; suitable for food, cosmetics, daily chemicals, pharmaceuticals.
- Strict control of pesticides, heavy metals, microbes, solvent residues and cyanogenic glycosides, plus standardized processing and storage ensure full compliance for all populations (children and pregnant women under guidance).

II. Product Applications

Based on core actives (anthocyanins, total flavonoids, phenolic acids, vitamin C) and functions (antioxidant, anti-inflammatory, immune regulation, soothing, skin-soothing, natural coloring), applications are divided into four fields: pharmaceutical, cosmetic, food, industrial and others.

(I) Pharmaceutical Applications

1. Core Value

As a natural adjuvant raw material for immune regulation, respiratory infection relief, skin inflammation repair and anti-oxidative damage. Effective rate for respiratory infection adjuvant relief ≥88%, fever/cough/sore throat relief ≥82%, skin inflammation relief ≥78%, DPPH scavenging rate ≥90% (0.5 g/L). Lower side effects than synthetic drugs.

2. Mechanisms

- **Immune regulation**: Activates macrophages, lymphocytes, NK cells (100 μg/mL increases NK activity by 52.3%), regulates cytokines (IL-2, TNF-α).
- **Respiratory protection**: Phenolic acids inhibit influenza/RSV replication, relieve cough and sore throat.
- **Skin repair**: Promotes fibroblast proliferation, inhibits TNF-α/IL-6, accelerates wound healing and reduces inflammation.
- **Antioxidant**: Scavenges free radicals, inhibits lipid peroxidation, protects organs.

3. Recommended Dosage Forms

- **Oral**: Tablets, capsules, granules, oral liquids, syrups; 150/300 mg/unit; disintegration ≤30 min, dissolution ≥80% in 45 min.
- **External**: Ointments (vaseline:lanolin = 7:3), gels, lotions (10%–15% ethanol).

4. Application Solutions

- **Scenarios**: Adjuvant treatment for cold, flu, bronchitis; immune support; skin inflammation/repair; sore throat mouthwash.
- **Compatible**: Astragalus extract, vitamin C, honeysuckle, forsythia, dipotassium glycyrrhizinate, panthenol.
- **Incompatible**: Immunosuppressants, spicy herbs, high-concentration acids, strong oxidizers.
- **Claims**: “Adjuvant immune enhancement”, “Adjuvant relief of respiratory symptoms”, “Adjuvant skin repair”; label “Not a replacement for prescription drugs”.

(II) Cosmetic Applications

1. Core Value

Powerful antioxidant, soothing, brightening, anti-inflammatory repair and mild moisturizing. DPPH scavenging ≥90% (0.5 g/L), better than vitamin C (65.2%); stratum corneum moisture ↑≥30% (0.5%); redness/stinging relief ≥88%. Natural purple colorant.

2. Mechanisms

- **Antioxidant**: Scavenges ROS, reduces oxidative damage, anti-aging, brightening.
- **Soothing & stabilizing**: Inhibits inflammatory factors, repairs barrier, enhances tolerance.
- **Anti-inflammatory repair**: Inhibits *Cutibacterium acnes*, repairs barrier damage and sunburn.
- **Moisturizing**: Polysaccharides form breathable film, reduce water loss.
- **Natural coloring**: Anthocyanins provide stable purple hue.

3. Recommended Dosage Forms

- **Liquids**: Toners, essences; pH 4.5–6.5, addition ≤40°C.
- **Emulsions**: Lotions, creams; O/W for normal skin, W/O for dry skin.
- **Others**: Masks, gels, lipsticks, sunscreens.

4. Application Solutions

- **Scenarios**: Sensitive skin care, brightening, anti-aging, acne, sunburn repair, lip care, natural coloring.
- **Compatible**: Licorice extract, hyaluronic acid, vitamin C, retinol, glycerin, ceramides, panthenol.
- **Incompatible**: High-concentration acids (≥5% AHA), strong oxidizers, formaldehyde-releasers, high alcohol (≥20%).
- **Claims**: “Powerful antioxidant”, “Brighten skin tone”, “Soothe redness”, “Repair barrier”, “Mild moisturizing”, “Natural coloring”.

(III) Food Applications

1. Core Value

Natural antioxidant, coloring, functional conditioning and flavor enhancement. Replaces 30%–50% synthetic antioxidants (BHA, BHT) and chemical pigments. Lipid peroxidation inhibition ≥75% (0.1 g/L).

2. Mechanisms

- **Antioxidant**: Scavenges free radicals, inhibits oil oxidation, extends shelf life.
- **Natural coloring**: Stable purple hue for beverages, confectionery, baked goods.
- **Functional**: Immune support, respiratory relief, gastrointestinal protection.
- **Flavor**: Mild fruity aroma, reduces artificial flavors.

3. Recommended Dosage Forms

- **Liquids**: Juices, teas, oral liquids, dairy drinks, wines; addition ≤60°C.
- **Solids**: Baked goods, nuts, candies, health food tablets, meal replacements; microencapsulation recommended (embedding rate ≥85%).
- **Oils**: Edible oils, fried foods; direct powder addition.

4. Application Solutions

- **Scenarios**: Oil preservation, baked goods, beverages, health foods (immune, respiratory), confectionery.
- **Compatible**: Vitamin E, tea polyphenols, probiotics, dietary fiber, mint, tangerine peel, beet red.

- **Incompatible**: High-concentration acids (≥10% citric acid), strong flavor additives, immunosuppressants.
- **Claims**: "Natural antioxidant", "Extend shelf life", "Natural coloring" for ordinary food; "Adjuvant immune enhancement" for health foods (label "Not a drug").

(IV) Industrial and Other Applications

1. Core Value

Green environmental protection, natural coloring and performance improvement. Replaces synthetic additives. 1%–5% for wood preservation → decay fungi mortality ≥90%; 0.5% for textile/leather dyeing → washing fastness ≥3 grade; replaces 20%–30% antibiotics in feed.

2. Mechanisms

- **Preservation**: Inhibits microbes and insects for wood/leather.
- **Coloring**: Stable purple for textiles, leather, plastics.
- **Antioxidant**: Delays aging of rubber, plastics.
- **Feed support**: Improves animal immunity and intestinal health.
- **Leather/textile conditioning**: Enhances flexibility, gloss, antistatic property.

3. Recommended Dosage Forms

Solutions, suspensions, premixes, powders for wood preservation, leather, textiles, feed, rubber, plastics, wastewater treatment.

4. Application Solutions

- **Scenarios**: Wood preservation; leather tanning/coloring; wastewater purification; textile dyeing; green feed additives; rubber/plastic anti-aging; cosmetic packaging preservation/coloring.
- **Compatible**: Boric acid, chromium salts, alum, probiotics, vitamin E.
- **Incompatible**: Strong oxidants, high-concentration alkalis (≥5% NaOH), heavy metal salts (except chromium), strong reducing agents.
- **Claims**: "Green preservation", "Natural environmental protection", "Natural coloring", "Extend service life", "Green feed additive".

III. Recommended Dosage

Based on conventional purity: anthocyanins 3.0%–5.0%, total flavonoids 2.0%–3.0%, phenolic acids 1.0%–2.0%. Adjust proportionally for higher purity.

Core Dosage Summary by Field

- **Pharmaceutical**:

Oral: 150–300 mg/tablet/capsule, daily 300–900 mg (as anthocyanins);

External: 2.0%–8.0% (ointment/gel), 1.0%–5.0% (lotion), 0.5%–2.0% (mouthwash);

Syrups/oral liquids: 1.0%–3.0%.

- **Cosmetic**:

Leave-on: 0.01%–0.5% (0.01%–0.1% for sensitive skin, ≤1.0% for infants);

Rinse-off: 0.1%–2.0% (max 4.0%);

Sunscreen: 0.5%–1.0%;

Lipsticks/gels: 5.0%–15.0%;

Color cosmetics: 0.5%–3.0%.

- **Food**:

Antioxidant: 0.01%–0.1% (oils 0.05%–0.1%, beverages 0.01%–0.05%);

Coloring: 0.05%–0.5% (beverages 0.05%–0.2%, confectionery/baked goods 0.1%–0.5%);

Preservative: 0.05%–0.5%;

Health food: 0.5%–2.0% (daily intake ≤500 mg as anthocyanins).

- **Industrial**:

Wood preservation: 1%–5% (solution);

Leather: 0.5%–2.0% (3%–8% for coloring);

Chemical: 0.1%–1.0% (antioxidant), 1%–10% (adsorbent);

Textile: 1%–5% (dye), 0.1%–0.5% (additive);

Feed: 0.1%–0.5% (10%–20% premix);

Wastewater: 5%–10% (solution);

Rubber/plastics: 0.1%–0.5%.

IV. Supplementary Notes

- **Purity**: Higher purity allows lower dosage; select products complying with *Chinese Pharmacopoeia* or ISO 23311:2022; black elderberry has higher anthocyanins.
- **Storage**: Sealed, away from light, cool and dry, shelf life ~24 months; use within 1 month after opening; avoid high temperature and strong light.
- **Testing**: Heavy metals, microbes, pesticides, cyanogenic glycosides, anthocyanins, flavonoids must be tested batchwise.
- **Special populations**: Pregnant/lactating women, children, gastrointestinal-sensitive people use under guidance; prohibit use by allergic individuals.
- **Processing**: Control temperature and pH; avoid strong acids/alkalis; prefer solvent-free extraction; microencapsulation improves stability.