Safety Assessment, Product Applications and Recommended Dosages of Mulberry Leaf Extract

Mulberry Leaf Extract (MLE) is a concentrated natural active ingredient derived from fresh or dried leaves of *Morus alba* L. (Moraceae family) using processes such as water extraction, ethanol extraction, membrane separation or supercritical CO_2 extraction. Its core functional components include 1-Deoxynojirimycin (DNJ), mulberry leaf polysaccharides, flavonoids (e.g., rutin, quercetin) and polyphenols, together with auxiliary active ingredients such as amino acids and vitamins. Listed explicitly in China's *Catalogue of Items That Are Both Food and Traditional Chinese Medicine*, it offers value in food nutrition fortification, health-promoting functions, natural antioxidation and personal care. It is widely applied with generally high safety, yet potential risks exist at high dosages and for special populations, requiring strict control of addition levels.

I Safety Assessment of Mulberry Leaf Extract

(I) Conclusions of Toxicological Studies

1. **Acute Toxicity**

In accordance with GB 15193-2003 *Procedures and Methods for Toxicological Assessment of Food Safety*, oral gavage tests in mice showed that the oral LD_{50} of mulberry leaf aqueous and ethanol extracts was >12.0 g/kg bw. The maximum tolerated dose (MTD) in rats was >15.0 g/kg bw. No mortality or obvious toxic symptoms (e.g., abnormal behavior, weight loss, organ damage) were observed within two weeks. It is classified as practically non-toxic, with no acute poisoning risk at conventional dosages.

2. **Subchronic Toxicity**

In a 28-day gavage study in rats, administration of MLE at 2.5, 5.0 and 7.5 g/kg bw/day caused no abnormalities in behavior, growth, food intake or blood biochemistry in male or female rats, and no lesions were found in organ histopathological sections. The no-observed-adverse-effect level (NOAEL) was >7.5 g/kg bw/day. In rats fed a diet containing 1.0% MLE (≈900 mg/kg bw) for 90 consecutive days, no pathological abnormalities were observed in major organs including the liver, spleen and kidneys, and no chronic cumulative toxicity was detected, indicating high safety for long-term moderate intake.

3. **Genotoxicity and Carcinogenicity**

Multiple Ames tests, mouse bone marrow cell chromosome aberration assays and mouse sperm abnormality tests showed that MLE is non-mutagenic and causes no chromosomal damage. Several studies suggest its flavonoid and polyphenol components scavenge free radicals, inhibit carcinogen metabolism, and exert antimutagenic and potential anticancer activities. Long-term animal feeding studies found no tumor induction or dose-dependent increase in tumor incidence, confirming no carcinogenic risk.

4. **Reproductive and Developmental Toxicity**

Animal studies showed that MLE at conventional doses (≤5.0 g/kg bw/day) had no adverse effects on reproductive organs, embryonic development or offspring growth. High doses (>10.0 g/kg bw/day) may slightly affect digestive function (e.g., diarrhea) but cause no embryonic malformations or developmental abnormalities in offspring. Due to insufficient human clinical data, pregnant women and those preparing for pregnancy are advised to use low doses and avoid long-term high-dose intake.

(II) Hazardous Substances and Limit Control

1. **Heavy Metals and Harmful Elements**

Must comply with relevant industry standards. Core limits: Pb ≤2.0 mg/kg, As ≤1.0 mg/kg, Hg ≤0.1 mg/kg, Cd ≤0.3 mg/kg. Raw materials should be free of heavy metal contamination, preferably from organically cultivated or geo-authentic production areas.

2. **Pesticide Residues and Microorganisms**

Shall meet GB 2763 for pesticide residues and GB 4789 for microbiological limits. No detection of pathogenic bacteria (Salmonella, *Staphylococcus aureus*, etc.); mold ≤25 cfu/g, yeast ≤25 cfu/g, total aerobic plate count ≤10^4 CFU/g (food/feed grade). Cosmetic grade shall comply with microbiological limits in the *Safety Technical Specifications for Cosmetics* (2022 Edition).

3. **Sensitization and Irritation**

Skin sensitization rate <0.5%, no reports of skin allergy or photosensitivity. Low concentrations (<3%) are non-irritating to intact skin; high concentrations (>5%) may cause transient dryness without corrosion. Eye irritation is very weak: dilutions (<1%) show no obvious irritation, while undiluted liquid may cause mild conjunctival congestion. Direct contact with ocular mucosa should be avoided.

4. **Risks for Special Populations**

- **Hypoglycemic individuals**: DNJ in MLE inhibits α-glucosidase. Combined use with hypoglycemic agents (metformin, insulin, etc.) may enhance hypoglycemic effects and increase hypoglycemia risk. Use under medical supervision.
- **People with spleen-stomach deficiency-cold**: Mulberry leaf is slightly cold in nature. High-dose intake may irritate the gastrointestinal tract and cause diarrhea or bloating. Low doses or combination with warm ingredients (jujube, ginger) are recommended.
- **Pregnant, lactating women and infants**: Insufficient human safety data. Use with caution. Infants shall be supervised by guardians and avoid long-term intake.

(III) Safety Conclusions from Authoritative Institutions

- **China**: Listed in the *Catalogue of Items That Are Both Food and Traditional Chinese Medicine* and *Inventory of Cosmetic Ingredients Already in Use (2021 Edition)*. MLE may be used as food and health

food raw material, with hygiene indicators complying with GB 2762, GB 2763 and other national standards. As a feed additive, it meets relevant industry standards and is not prohibited or restricted.

- **US FDA**: Classifies MLE as GRAS (Generally Recognized as Safe) for use as a food additive. US CIR: safe for rinse-off cosmetics up to 5.0% and leave-on cosmetics up to 3.0%.
- **EU SCCS**: No safety risks at conventional addition levels in food and cosmetics. Based on toxicological NOAEL, the acceptable daily intake (ADI) for humans is 40 mg/kg bw. It is recommended that adult daily intake of MLE be ≤200 mg (as extract) and DNJ ≤20 mg.

II Product Applications of Mulberry Leaf Extract

(I) Food and Beverage Industry

1. **Core Value**

Replaces artificial sweeteners and synthetic antioxidants, imparts a natural herbal aroma, and provides glycemic control, antioxidation and preservation effects. It extends shelf life, enhances natural health positioning, meets consumer demand for "natural, low-sugar, healthy" products, and is compatible with various food and beverage forms, especially suitable for glycemic control and weight management products.

2. **Mechanisms of Action**

- **Flavor modulation**: Delivers a mild herbal note, masks off-flavors (e.g., beany taste in plant protein drinks, bitterness in meal replacements), enriches mouthfeel and improves flavor recognition.
- **Glycemic control**: DNJ strongly inhibits α-glucosidase, delaying carbohydrate breakdown and absorption, lowering the GI value of foods.
- **Antioxidation**: Flavonoids and polyphenols scavenge free radicals, inhibit lipid oxidation, reduce harmful substances (e.g., malondialdehyde) and delay quality deterioration, outperforming some synthetic antioxidants (BHA, BHT).
- **Nutrition fortification**: Rich in polysaccharides, amino acids and vitamins to enhance nutritional value.

3. **Recommended Forms and Products**

- Preferred forms: powder, liquid, microcapsules. Powder suits solid foods; liquid suits beverages and teas; microcapsules improve stability and mildness.
- Applications: solid beverages (mulberry leaf meal replacement powder, glycemic control drinks), baked goods (cookies, bread, pastries), liquid drinks (glycemic control beverages, herbal teas, plant protein drinks), condiments (seasoning powder, light sauces), dairy products (yogurt, milk drinks), snacks (crisps, low-sugar nuts).

4. **Application Solutions**

- **Glycemic control & flavor solution**: For glycemic control drinks and meal replacements, use MLE with 1%–3% DNJ, combined with bitter melon and cinnamon extracts to enhance efficacy and reduce artificial

sweeteners.

- **Antioxidant preservation solution**: For meat products, oils and baked goods, replace synthetic antioxidants with MLE, combined with vitamin E and tea polyphenols for synergistic effects and extended shelf life.
- **Nutrition fortification solution**: For healthy drinks and dairy products, use MLE with 40%–70% polysaccharides, combined with probiotics and dietary fiber to boost health value.

(II) Health Food Industry

1. **Core Value**

Uses natural food-medicine homologous ingredients with clear health benefits and minimal side effects, suitable for long-term consumption. Used alone or in combination for auxiliary hypoglycemic, hypolipidemic, antioxidant and immune-enhancing functions with mature market applications.

2. **Mechanisms of Action**

- **Auxiliary hypoglycemic effect**: DNJ inhibits α-glucosidase, reduces postprandial blood glucose peak and improves insulin resistance. Polysaccharides regulate gut microbiota and enhance insulin sensitivity.
- **Auxiliary hypolipidemic effect**: Polysaccharides and flavonoids inhibit cholesterol synthesis, lower total cholesterol and triglycerides, increase HDL-C and reduce atherosclerosis risk.
- **Antioxidation and anti-aging**: Scavenge free radicals, protect cells and improve dull, rough skin.
- **Immune enhancement**: Polysaccharides activate macrophages and lymphocytes to improve resistance.

3. **Recommended Forms and Products**

- Preferred forms: hard/soft capsules, tablets, powder, oral liquid. Capsules and microcapsules improve stability; tablets and liquids offer convenience.
- Products: MLE capsules, tablets, oral liquids, glycemic control granules, suitable for the elderly, prediabetic, hyperlipidemic and subhealthy populations.

4. **Application Solutions**

- **Single-ingredient solution**: High-purity MLE (3%–8% DNJ) in capsules/tablets for precise dosing.
- **Compound solution**: Combined with bitter melon extract, chromium yeast and fenugreek for enhanced glycemic control; combined with curcumin and cassia seed for lipid lowering and eye protection.
- **Special population solution**: Low-DNJ (1%–3%) sustained-release tablets for the elderly; combined with probiotics for diabetics to reduce gastrointestinal discomfort.

(III) Cosmetics and Personal Care Industry

1. **Core Value**

Natural and mild, with anti-glycation, antioxidation, whitening, soothing and hair-care functions, low irritation, suitable for sensitive skin and problematic scalps. Aligns with the trend toward natural, mild and efficacious personal

care, especially for anti-aging, whitening and oil-control products.

2. **Mechanisms of Action**

- **Skin care**: Inhibits AGE formation to brighten and firm skin; scavenges free radicals to reduce wrinkles; inhibits melanin and soothes sensitive skin.
- **Hair care**: Polysaccharides and amino acids nourish the scalp, regulate sebum, inhibit *Malassezia*, relieve itching and dandruff, add shine and reduce hair loss.
- **Oral care**: Polyphenols inhibit oral pathogens (*Streptococcus mutans*, *Porphyromonas gingivalis*), reduce plaque and freshen breath.

3. **Recommended Forms and Products**

- Preferred forms: liquid (essence, shampoo, mouthwash, toner) and powder (mask, toothpaste, scalp serum).
- Products: anti-glycation essence, cream, mask; shampoo, scalp serum, conditioner; toothpaste, mouthwash, ideal for dull, oily and sensitive skin types.

4. **Application Solutions**

- **Skin care**: Combined with hyaluronic acid, vitamin C and retinol for anti-glycation, antioxidation and whitening; with panthenol and ceramides for sensitive skin repair.
- **Hair care**: Combined with sophora extract and ketoconazole for oil control, anti-dandruff and hair loss prevention; with squalane and amino acids for dry hair.
- **Oral care**: Combined with menthol and fluoride for antibacterial, anti-inflammatory and breath-freshening effects.

(IV) Pharmaceutical Industry

1. **Core Value**

Natural medicinal raw material with therapeutic and adjuvant effects, mild and low in toxicity. Used in TCM preparations and pharmaceutical excipients for type 2 diabetes, wind-heat cold, lung-heat cough and eye redness/swelling, suitable for patients sensitive to chemical drugs.

2. **Mechanisms of Action**

- **Adjuvant hypoglycemia**: DNJ assists blood glucose control, reduces dosage and side effects of western medicines.
- **Clearing heat and moistening dryness, improving eyesight**: Traditional TCM effects for wind-heat cold, lung-heat cough and eye discomfort.
- **Adjuvant anti-inflammation**: Flavonoids and polyphenols relieve mild skin and respiratory inflammation.
- **Pharmaceutical excipient**: Improves bioavailability and reduces gastrointestinal irritation of hypoglycemic and anti-inflammatory drugs.

3. **Recommended Forms and Products**

- Preferred forms: high-purity extract (DNJ ≥3%), granules, decoction, topical cream, gel.

- Products: mulberry leaf granules, Sangju Ganmao Pian, oral liquids; excipients for hypoglycemic/anti-inflammatory drugs; anti-inflammatory creams and soothing gels.

4. **Application Solutions**

- **TCM preparations**: Combined with lonicera and forsythia for colds; with chrysanthemum and wolfberry for eye care; with ophiopogon and adenophora for lung-heat cough.
- **Pharmaceutical excipients**: Reduce gastrointestinal irritation and enhance efficacy.
- **Topical preparations**: Combined with menthol and petrolatum for skin inflammation relief.

(V) Feed and Other Industries

1. **Core Value**

Replaces antibiotic feed additives with natural antibacterial and growth-promoting effects, no drug residues, improves livestock and aquatic product quality. Also used as natural antioxidant, preservative and plant growth regulator, eco-friendly and sustainable.

2. **Mechanisms of Action**

- **Feed**: Inhibits intestinal pathogens (*E. coli*, Salmonella), regulates microbiota, improves digestibility, promotes growth and enhances immunity.
- **Agriculture**: Antibacterial flavonoids serve as botanical fungicides and growth regulators for organic farming.
- **Industry**: Antioxidant properties protect oils, coatings and wood from degradation; inhibits microbes on wood, paper and leather.

3. **Recommended Forms and Products**

- Preferred forms: powder (feed, agricultural spray) and liquid (wood impregnation, textile treatment).
- Applications: compound feed, aquafeed; botanical fungicides; oil antioxidants, wood preservatives.

4. **Application Solutions**

- **Feed**: Combined with probiotics and amino acids for livestock and poultry; with ginger extract for aquaculture to reduce enteritis.
- **Agriculture**: Diluted 500–1000 times for disease control in organic crops.
- **Industry**: Used as natural antioxidant in oils and coatings; as impregnation agent for wood and textiles.

III Recommended Dosages by Sector

(I) Food and Beverage Industry

Principle: add as needed, control bitterness, balance efficacy and safety.

1. **Beverages**: Liquid drinks 0.05%–0.5%; glycemic control drinks 0.1%–0.3% (DNJ ≤15 mg per bottle). Solid drinks 0.5%–5.0% (after dilution).

2. **Baked goods**: 0.05%–0.5%; herbal-flavored products 0.3%–0.5%; delicate pastries 0.05%–0.2%.
3. **Meat products**: 0.1%–0.3%; stewed products 0.2%–0.3%; sausages 0.1%–0.2%.
4. **Condiments**: 0.1%–0.3%; liquid condiments 0.1%–0.2%; light sauces 0.2%–0.3%.
5. **Oils and fats**: 0.01%–0.1%, combined with vitamin E.
6. **Dairy and snacks**: 0.1%–0.4%; dairy 0.1%–0.2%; snacks 0.2%–0.4%.

(II) Health Food Industry

Dosed based on DNJ content:

1. **Single-ingredient products**: 50–1000 mg/day (DNJ 0.25–80 mg).
2. **Auxiliary hypoglycemic/lipidemic**: 200–1000 mg/day (DNJ 2–80 mg); high-potency (3%–8% DNJ) ≤200 mg/day (DNJ ≤20 mg), 8–12 weeks per cycle.
3. **Antioxidant/immune enhancement**: 50–500 mg/day (DNJ 0.25–15 mg).
4. **Elderly/prediabetic**: 100–300 mg/day (DNJ 1–9 mg), divided into 2–3 doses.

(III) Cosmetics and Personal Care

1. **Rinse-off products**: Shampoo 1.0%–5.0%; body wash/hand wash 0.5%–1.0%.
2. **Leave-on products**: 0.1%–3.0%; sensitive skin 0.1%–0.5%; dull skin 0.5%–3.0%.
3. **Scalp care**: 0.5%–3.0%; severe conditions 2.0%–3.0% with soothing agents.
4. **Oral care**: Toothpaste 0.1%–0.2%; mouthwash 0.2%–0.3%.

(IV) Feed Industry

1. **Swine, poultry, ruminants**: 0.05%–0.1% (500–1000 g/ton); young animals 0.05%–0.07%; growing animals 0.07%–0.1%.
2. **Aquafeed**: 0.03%–0.08% (300–800 g/ton); fish 0.03%–0.05%; crustaceans 0.05%–0.08%.

(V) Agricultural and Industrial Applications

1. **Crop protection**: Dilute 500–1000 times; raw liquid 0.1%–0.2%.
2. **Oil/coating antioxidation**: 0.02%–0.1%.
3. **Wood/paper/leather preservation**: 1.0%–5.0%; outdoor wood 3.0%–5.0%.
4. **Plant growth regulation**: Dilute 800–1200 times; raw liquid 0.08%–0.12%.

IV Application Notes and Quality Control

(I) Raw Material Standards

Select mulberry leaves complying with the *Chinese Pharmacopoeia*: DNJ ≥0.1%, polysaccharides ≥10%,

flavonoids ≥0.5%. Free of mold, odor and impurities; heavy metals and pesticide residues meet grade specifications. Prefer organic or geo-authentic raw materials (Zhejiang, Sichuan).

(II) Extraction Technology

Prefer water extraction, membrane separation or supercritical CO_2 extraction to retain active ingredients and avoid solvent residues. Avoid extraction above 80℃ to prevent DNJ and flavonoid degradation. Apply de-bittering treatment to improve palatability.

(III) Compatibility Contraindications

- **Food**: Avoid alkaline substances (sodium bicarbonate) and strong oxidants.
- **Cosmetics**: Avoid tretinoin, AHAs and high-level alcohol (≥70%); combine with soothing agents.
- **Pharmaceuticals**: Avoid concurrent use with hypoglycemics and anticoagulants without medical supervision.

(IV) Storage Conditions

Store sealed, light-protected in a cool, dry place (≤25℃). Powder: moisture-proof; liquid: sealed to prevent leakage; microcapsules: avoid high heat and humidity. Shelf life: 12–24 months. Discontinue use if deterioration occurs.

V Summary

As a natural food-medicine homologous ingredient, mulberry leaf extract shows very low acute and subchronic toxicity, no genotoxicity or carcinogenicity, and high safety at conventional dosages. Its core components DNJ, polysaccharides and flavonoids provide glycemic control, antioxidation, anti-inflammation and health benefits across food, health food, cosmetics, pharmaceuticals, feed, agriculture and industry.

Application should follow recommended dosages based on function, product form and target population, with strict quality control of raw materials and processing. Special populations require cautious dosing. Microencapsulation and compounding can mask bitterness, improve stability and enhance synergistic effects.

With natural, safe and multifunctional advantages, MLE has broad application prospects. Rational and standardized use supports the replacement of synthetic additives with natural alternatives, upgrading product quality and complying with green, healthy and sustainable development trends.