

Apigenin and Eupatilin: A Pharmacognostic and Pharmacological Perspective

Pooja Bharati¹

1. Faculty of Pharmaceutical Sciences, Jayoti Vidyapeeth Women's University, Jaipur 303122

Abstract:

Apigenin (4',5,7-trihydroxyflavone) and Eupatilin (5,7-dihydroxy-3',4',6-trimethoxyflavone) are naturally occurring flavones with broad pharmacological significance. They are primarily obtained from *Matricaria chamomilla*, *Petroselinum crispum*, *Citrus aurantium*, and *Artemisia asiatica*. Both compounds possess diverse biological activities, including antioxidant, anti-inflammatory, anticancer, neuroprotective, hepatoprotective, and gastroprotective effects. This chapter presents a detailed pharmacognostic, phytochemical, and pharmacological profile of Apigenin and Eupatilin, focusing on their structural characteristics, molecular mechanisms, and therapeutic relevance. The pharmacognostic section discusses botanical sources, morphological features, and optimized extraction and isolation procedures involving ethanol, methanol, and chromatographic purification. Phytochemical characterization includes spectroscopic data such as UV, IR, NMR, and MS confirming hydroxyl and methoxy functional groups. Quantitative estimation through HPLC and HPTLC ensures standardization and quality control of plant extracts containing these flavones. Apigenin acts by modulating NF- κ B, STAT3, and MAPK pathways, leading to the suppression of pro-inflammatory mediators, inhibition of tumor cell proliferation, and enhancement of neuronal protection. Eupatilin mediates its effects primarily through inhibition of COX-2, TGF- β , and activation of the Nrf2/HO-1 antioxidant system, contributing to gastroprotection and cytoprotection against oxidative damage. Both compounds exhibit significant free radical scavenging and anti-apoptotic activities in preclinical models. Pharmacokinetic studies reveal limited solubility and low oral bioavailability due to extensive glucuronidation and sulfation. Novel drug delivery approaches such as nanoparticles, liposomes, and cyclodextrin inclusion complexes have improved their pharmacokinetic profiles. Toxicological data confirm high safety margins without organ-specific toxicity. Recent research highlights advances in nanoformulations, synergistic applications, and targeted delivery systems. Despite promising preclinical results, clinical validation remains limited, emphasizing the need for further mechanistic and pharmacokinetic optimization. The comprehensive pharmacognostic and pharmacological evaluation presented in this chapter supports Apigenin and Eupatilin as potential lead molecules for future phytopharmaceutical and therapeutic development.

Keywords: Apigenin, Eupatilin, Flavones, Pharmacognosy, Phytochemistry, Antioxidant, Anti-inflammatory, Pharmacokinetics, Nanotechnology, Drug delivery, Phytopharmaceuticals

Corresponding Author

Pooja Bharati

Faculty of Pharmaceutical Sciences, Jayoti Vidyapeeth Women's University, Jaipur 303122

pooja7905059216@gmail.com

Table of Contents

Introduction

Pharmacognostic Overview

Source and Occurrence

Botanical Description of Source Plants

Extraction and Isolation Techniques

Pharmacological Activities of Apigenin

Antioxidant Activity

Anti-inflammatory and Analgesic Effects

Anticancer Potential

Neuroprotective and Cognitive Effects

Cardioprotective and Antidiabetic Actions

Other Reported Pharmacological Activities

Pharmacological Activities of Eupatilin

Anti-ulcer and Gastroprotective Activity

Anti-inflammatory and Antioxidant Properties

Anticancer and Chemopreventive Roles

Neuroprotective and Hepatoprotective Effects

Other Pharmacological Findings

Future Prospects and Research Gaps

References

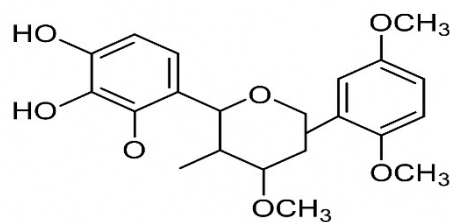
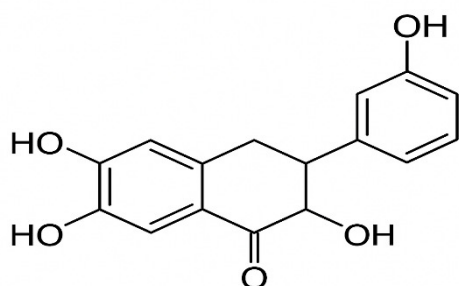
Introduction

Flavonoids are a major class of naturally occurring polyphenolic compounds widely distributed in fruits, vegetables, and medicinal plants. They play essential roles in plant physiology and contribute to the therapeutic properties of numerous herbal preparations. Among various subclasses, *flavones* have drawn particular interest due to their diverse pharmacological activities and structural stability. Two notable flavones—**Apigenin (4',5,7-trihydroxyflavone)** and **Eupatilin (5,7-dihydroxy-3',4',6-trimethoxyflavone)**—have been extensively investigated for their potential in disease prevention and treatment. Both compounds are secondary metabolites with promising pharmacological profiles supported by experimental and limited clinical data.

Apigenin occurs abundantly in *Matricaria chamomilla* (chamomile), *Petroselinum crispum* (parsley), *Apium graveolens* (celery), *Ocimum basilicum* (basil), and *Citrus aurantium* (orange peel). It contributes to the traditional medicinal use of chamomile as an anti-inflammatory and sedative agent. Eupatilin, on the other hand, is mainly isolated from *Artemisia asiatica* and *Artemisia princeps*, species long used in East Asian medicine for treating gastric disorders, inflammation, and oxidative stress. The abundance of both compounds in edible and medicinal plants makes them attractive candidates for nutraceutical and pharmaceutical development.

Chemically, Apigenin and Eupatilin share a common flavone backbone consisting of two aromatic rings (A and B) linked through a heterocyclic pyrone ring (C). The variation in hydroxyl and methoxy substituents defines their biological activity and solubility. Apigenin possesses three hydroxyl groups at positions 4', 5, and 7, while Eupatilin contains two hydroxyls (5 and 7) and three methoxy groups (3', 4', 6). These structural features influence lipophilicity, membrane permeability, and interaction with biomolecular targets such as enzymes and receptors.

Apigenin



Eupatilin

Figure: Chemical Structure of Apigenin & Eupatilin

Over the past two decades, research on Apigenin and Eupatilin has expanded across multiple pharmacological areas. Both exhibit **strong antioxidant activity**, scavenging reactive oxygen species (ROS) and enhancing endogenous defense enzymes such as superoxide dismutase and catalase. **Anti-inflammatory properties** are well established through inhibition of key mediators like COX-2, iNOS, NF- κ B, and TNF- α . **Anticancer effects** have been demonstrated in diverse cancer cell lines, where both flavones induce apoptosis, arrest cell cycle progression, and suppress angiogenesis. **Neuroprotective actions** involve modulation of oxidative stress, cholinergic function, and inflammatory signaling, suggesting potential use in neurodegenerative diseases like Alzheimer's and Parkinson's. Additionally, **Eupatilin** shows marked **gastroprotective activity**, reducing mucosal injury and oxidative stress in experimental ulcer models.

Despite their potent pharmacological effects, both compounds face challenges related to **low water solubility**, **limited bioavailability**, and **rapid metabolism** through glucuronidation and sulfation. These limitations have driven research into advanced delivery systems, including polymeric nanoparticles, liposomes, solid lipid carriers, and cyclodextrin inclusion complexes. Such strategies enhance stability, absorption, and systemic exposure, improving therapeutic potential.

This chapter compiles a detailed review of Apigenin and Eupatilin covering pharmacognostic features, phytochemical characterization, pharmacological activities, mechanisms of action. It integrates traditional knowledge with modern scientific evidence to highlight their clinical relevance and future applications. By exploring their chemistry in a unified framework, the chapter aims to establish Apigenin and Eupatilin as model flavones for phytopharmaceutical development and rational drug design.

Botanical Description of Source Plants

Botanical characterization is the first and most critical step in pharmacognostic identification. Accurate identification ensures the authenticity and purity of the plant material used in pharmacological and pharmaceutical studies. Morphological, anatomical, and microscopic features serve as diagnostic markers that differentiate genuine drug sources from adulterants.

This section outlines the detailed botanical characteristics of two major Apigenin-rich and Eupatilin-rich plants:

Matricaria chamomilla L. (German Chamomile) – primary source of Apigenin**Artemisia asiatica Nakai – primary source of Eupatilin**

Each plant is discussed under taxonomy, morphology, macroscopic and microscopic characters, and diagnostic pharmacognostic features.

Taxonomic Classification

Taxonomic Rank	Matricaria chamomilla L.	Artemisia asiatica Nakai
Kingdom	Plantae	Plantae
Division	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida
Order	Asterales	Asterales
Family	Asteraceae	Asteraceae
Genus	Matricaria	Artemisia
Species	M. chamomilla L.	A. asiatica Nakai
Common Name	German Chamomile	Asian Mugwort
Vernacular Name (Hindi)	Babuna	Nagdona / Damanaka

Both belong to Asteraceae, one of the largest families of flowering plants known for its rich flavonoid profile, particularly flavones and flavonols.

Macroscopic (Morphological) Description

A. Matricaria chamomilla L. (German Chamomile)
Habit: Annual herb, aromatic, erect, 15–60 cm tall.
Stem: Cylindrical, hollow, smooth, green, highly branched.
Leaves: Alternate, sessile, bipinnate or tripinnate, linear lobes, 2–5 cm long.
Inflorescence: Solitary capitulum (head) on long peduncles.
Flowers: Heterogamous—central tubular yellow disc florets (bisexual) and peripheral white ray florets (female).
Fruit: Cypsela, oblong, ribbed, without pappus.
Odor: Strong, apple-like fragrance due to volatile oils.
Taste: Slightly bitter, aromatic.

Diagnostic Features:

Hollow receptacle (distinguishes it from false chamomile, *Anthemis nobilis*), White ligulate ray florets turning downwards after blooming, Aromatic, sweet odor

B. <i>Artemisia asiatica</i> Nakai (Asian Mugwort)
Habit: Perennial herb, 30–100 cm tall, aromatic.
Stem: Erect, woody at base, angular, pubescent.
Leaves: Alternate, deeply lobed, upper surface dark green, lower surface silvery with fine hairs.
Inflorescence: Panicle of small heads.
Flowers: Yellowish-green tubular florets, all bisexual, surrounded by bracts with glandular trichomes.
Fruit: Small achene without pappus.
Odor: Strong camphoraceous aroma due to sesquiterpenes and volatile oils.
Taste: Bitter, pungent.
Diagnostic Features: Silvery underside of leaves with dense tomentose covering, Characteristic camphor-like smell, Narrow, elongated heads with glandular secretions

Microscopic Description

A. <i>Matricaria chamomilla</i> (Flower)
Epidermis: Uniseriate, covered with multicellular glandular trichomes (2–4 cells).
Receptacle: Parenchymatous, hollow center, containing oil glands.
Corolla: Composed of polygonal epidermal cells with wavy anticlinal walls.
Pollen Grains: Tricolporate, spherical (diameter 20–25 μm).
Vascular System: Collateral bundles surrounded by sclerenchyma.
Secretory Structures: Abundant secretory ducts containing volatile oil droplets (bisabolol, chamazulene precursors).

B. Matricaria chamomilla (Leaf)
Epidermis: Dorsiventral with thin cuticle.
Trichomes: Two types—unicellular conical and multicellular glandular.
Mesophyll: Differentiated into palisade and spongy parenchyma.
Vascular Bundles: Collateral, enclosed in parenchymatous sheath.
Calcium Oxalate Crystals: Absent.
Diagnostic Microscopy: Hollow receptacle and glandular trichomes distinguish genuine M. chamomilla from Anthemis cotula (which has solid receptacle and non-glandular trichomes).

B. Artemisia asiatica (Leaf)
Epidermis: Both surfaces possess numerous tomentose trichomes, imparting a silvery-white appearance.
Trichomes: Two main types:
T-shaped non-glandular hairs (protective)
Globular glandular trichomes (secreting volatile oils and flavones)
Mesophyll: Compact palisade cells with chloroplasts and interspersed secretory cavities.
Vascular Bundles: Collateral with lignified xylem and phloem parenchyma.
Secretory Canals: Present near vascular bundles containing Eupatilin-rich resin.
Diagnostic Microscopy: Presence of both glandular and T-shaped hairs, with large schizogenous secretory canals, is unique to A. asiatica.

Powder Microscopy

A. Matricaria chamomilla Powder
Fragments of parenchyma with oil globules.
Trichomes —multicellular glandular type.
Pollen grains spherical with smooth exine.
Yellow fragments of corolla tissue.
Vascular tissue with lignified xylem vessels.

B. Artemisia asiatica Powder
Trichomes (T-shaped, covering) and glandular hairs abundant.
Fragments of secretory canals with resin content.
Parenchyma cells with greenish-yellow pigment (flavone deposits).
Fibers thick-walled, elongated.

Organoleptic Evaluation

Parameter	Matricaria chamomilla	Artemisia asiatica
Color	Yellowish-white (flower heads)	Greenish-silver (leaves)
Odor	Pleasant, apple-like	Pungent, camphoraceous
Taste	Slightly bitter, aromatic	Strongly bitter, resinous
Texture	Soft, compressible	Tough, leathery

Histochemical Tests

Test	Reagent	Observation (M. chamomilla)	Observation (A. asiatica)	Inference
Flavonoids	Shinoda test (Mg + HCl)	Red coloration	Orange-red	Presence of flavones
Volatile oils	Sudan III stain	Orange droplets	Red-orange globules	Oil glands present
Tannins	Ferric chloride	Greenish-black	Brownish-black	Phenolic compounds
Lignin	Phloroglucinol + HCl	Pink-red	Red	Lignified tissue present
Calcium oxalate	HCl treatment	Negative	Negative	Absent

Diagnostic Characters Summary

Diagnostic Feature	Matricaria chamomilla	Artemisia asiatica
Hollow receptacle	Present	Absent

Diagnostic Feature	Matricaria chamomilla	Artemisia asiatica
Type of trichomes	Multicellular glandular	T-shaped non-glandular and glandular
Secretory canals	Abundant in receptacle	Prominent in leaves
Odor type	Fruity (bisabolol oil)	Camphoraceous (eucalyptol, borneol)
Main active flavone	Apigenin	Eupatilin
Family characteristics	Radiate capitulum, ligulate ray florets	Discoid capitulum, small tubular florets

Biosynthesis of Apigenin and Eupatilin

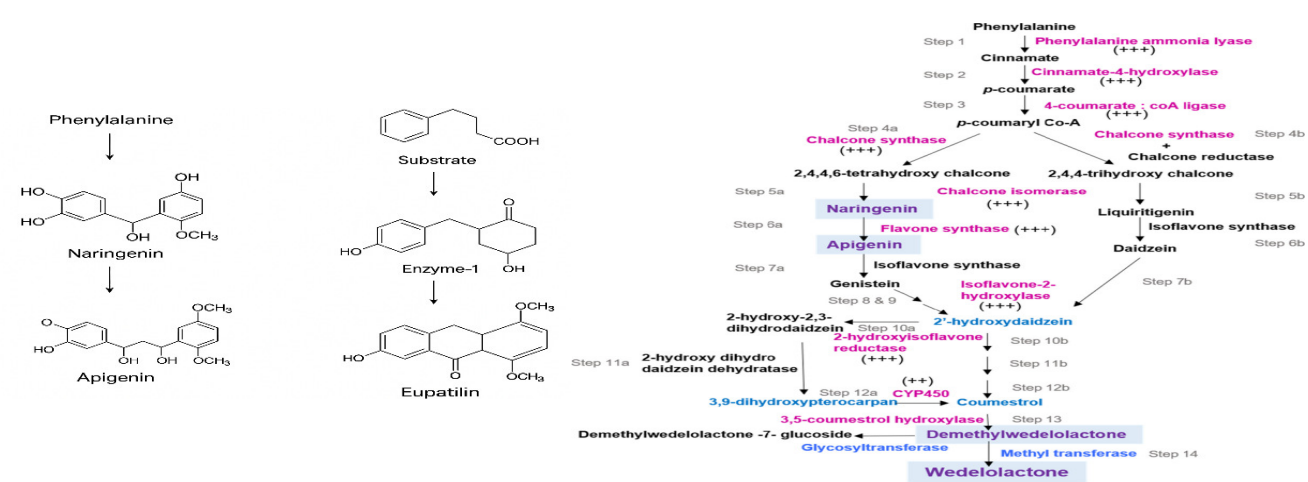


Figure: Biosynthesis of Apigenin and Eupatilin

Extraction and Isolation Techniques

Classical Extraction Methods and Principles

Extraction is a core pharmacognostic process that enables the isolation of bioactive compounds from complex plant matrices. For flavonoids such as apigenin and eupatilin, extraction efficiency and purity directly determine pharmacological reproducibility, standardization, and industrial scalability. The choice of extraction method depends on the nature of the compound, solvent polarity, plant matrix composition, and desired application. Classical solvent-based extraction methods such as maceration, percolation, Soxhlet extraction, and reflux remain the foundation of phytochemical studies and serve as benchmarks for evaluating advanced modern techniques.

Pre-extraction Preparation and Plant Material Standardization

Extraction begins with correct identification, authentication, and standardization of the source plant material. For apigenin, aerial parts of *Matricaria chamomilla*, *Petroselinum crispum*, *Apium graveolens*, *Citrus aurantium*, and *Thymus vulgaris* are commonly used, while eupatilin is isolated mainly from *Artemisia asiatica* and *Artemisia princeps*.

Freshly collected plant materials are cleaned to remove soil, debris, and foreign matter. Drying is carried out at controlled temperature (35–45°C) in a shade-drying chamber or oven with good airflow to prevent enzymatic degradation of flavonoid glycosides. The dried material is ground into a fine powder using a mechanical grinder and passed through a 40–60 mesh sieve to achieve uniform particle size, improving solvent penetration and extraction yield.

Moisture content, foreign organic matter, and extractive values are determined as per pharmacopoeial standards. The powdered drug is then stored in amber-colored, airtight containers at 25°C with less than 60% relative humidity. Prior to extraction, the powder may be defatted with petroleum ether (60–80°C) to remove nonpolar substances such as chlorophylls, waxes, and lipids that may interfere with subsequent purification steps.

Solvent Selection and Polarity Consideration

The selection of an appropriate solvent system is essential for efficient extraction. Apigenin and eupatilin are weakly acidic polyphenolic compounds with moderate lipophilicity, displaying poor solubility in water but good solubility in ethanol, methanol, acetone, ethyl acetate, and dimethyl sulfoxide. Solvent polarity determines both extraction yield and selectivity.

Table: Common solvents used for apigenin and eupatilin extraction

Solvent	Polarity Index	Typical Use	Remarks
Methanol	5.1	Universal flavonoid solvent	High extraction efficiency, toxic residue risk
Ethanol	4.3	Safe and eco-friendly	Preferred for food-grade and pharmaceutical extracts
Acetone	5.1	Intermediate polarity	Extracts flavonoid aglycones effectively
Ethyl acetate	4.4	Semi-polar solvent	Used in partitioning and fractionation
Water	9.0	Polar solvent	Used with co-solvents for glycoside extraction

Hydroalcoholic mixtures (ethanol-water or methanol-water) are commonly used to balance polarity and extract both aglycone and glycoside forms of flavonoids. Ethanol (70%) has been

found optimal for chamomile extraction, whereas 80% methanol is suitable for *Artemisia asiatica* eupatilin recovery.

Maceration Technique

Maceration is one of the oldest and simplest methods for flavonoid extraction. The powdered plant material is soaked in solvent at room temperature for a specified period (24–72 hours) with occasional stirring. This technique allows solvent molecules to diffuse through cell walls and dissolve intracellular constituents by concentration gradient.

For apigenin extraction, 100 g of powdered *Matricaria chamomilla* flowers are soaked in 1 L of 70% ethanol for 48 hours at 25°C. The extract is filtered and the residue re-macerated twice to ensure exhaustive extraction. Filtrates are combined and concentrated under reduced pressure at 40°C using a rotary evaporator. Maceration preserves thermolabile compounds but has low efficiency and longer duration. For eupatilin, maceration with methanol or acetone yields moderate concentrations, though prolonged exposure may lead to oxidation.

Advantages:

- Simple and low-cost
- Requires minimal equipment
- Suitable for thermolabile compounds

Disadvantages:

- Long extraction time
- Incomplete extraction without multiple cycles
- High solvent usage

Percolation

Percolation offers a continuous diffusion-based process suitable for large-scale extractions. In this technique, the powdered drug is moistened with solvent and packed in a conical percolator. Fresh solvent is continuously added, allowing the liquid extract (percolate) to drain from the bottom. The process continues until the percolate becomes nearly colorless.

Apigenin-rich extracts are often prepared by percolating parsley or chamomile powder with 70% ethanol. Percolation parameters such as solvent flow rate (1–3 mL/min), temperature (ambient), and percolation time (48–72 hours) are optimized to maximize yield. The percolate is filtered and concentrated.

For eupatilin, *Artemisia asiatica* aerial parts yield approximately 1.6% w/w eupatilin under optimized percolation conditions with 80% ethanol.

Advantages:

- Continuous operation improves mass transfer
- Suitable for industrial production
- Minimizes solvent degradation due to steady-state diffusion

Disadvantages:

- Requires close control of solvent flow
- Potential clogging in fine powders
- Less efficient for highly bound constituents

Soxhlet Extraction

Soxhlet extraction remains a gold standard for exhaustive extraction of flavonoids and other polyphenolics. In this method, the powdered material is placed in a thimble made of cellulose and continuously extracted with a volatile organic solvent under reflux. The solvent evaporates, condenses, and drips onto the sample, dissolving target compounds, which accumulate in the distillation flask.

For apigenin, Soxhlet extraction with 80% methanol or ethanol at 70°C for 6–8 hours yields up to 2.1% w/w extractive value. For eupatilin, methanol and acetone are preferred solvents, with yields ranging from 1.8% to 2.5% depending on the plant source.

Process Steps:

1. 50 g of dried plant powder is placed in a thimble.
2. 500 mL of solvent (methanol or ethanol) is added to the distillation flask.
3. Extraction is carried out at solvent reflux temperature (60–80°C).
4. After 6–8 hours (10–12 cycles), extraction is complete.
5. The extract is evaporated under reduced pressure to dryness.

Advantages:

- High efficiency and reproducibility
- Continuous solvent recycling minimizes loss
- Suitable for thermally stable compounds

Disadvantages:

- Not suitable for thermolabile constituents

- High energy consumption
- Requires long extraction time

Table: Comparison of classical extraction methods for apigenin and eupatilin

Method	Solvent	Temp (°C)	Time	Yield (%)	Key Advantages	Limitations
Maceration	70% Ethanol	25	48–72 h	1.2–1.6	Gentle extraction	Slow, incomplete
Percolation	70% Ethanol	25	48–72 h	1.5–1.8	Continuous diffusion	Requires flow control
Soxhlet	Methanol/Ethanol	70	6–8 h	2.0–2.2	Exhaustive extraction	Not for thermolabile compounds

Reflux Extraction

Reflux extraction is a hybrid of Soxhlet and maceration, using constant heating under reflux to speed up diffusion without solvent recycling. The drug is boiled with solvent (e.g., 80% ethanol) for 2–3 hours, cooled, and filtered. The process is repeated three times. This method has been used for extracting apigenin from parsley leaves and eupatilin from *Artemisia princeps*.

Reflux extraction at 80°C for 3 hours yields 1.9% apigenin with minimal degradation. For eupatilin, methanolic reflux gives a comparable yield with shorter duration than Soxhlet.

Advantages:

- Reduced extraction time compared to maceration
- Efficient solvent utilization
- Maintains moderate temperature control

Disadvantages:

- Risk of degradation at high temperature
- Limited automation potential

Filtration, Concentration, and Fractionation

After extraction, the filtrate is concentrated under reduced pressure using a rotary evaporator at temperatures below 45°C to prevent thermal decomposition. The concentrated extract is suspended in water and subjected to solvent–solvent partitioning for fractionation. Nonpolar impurities are removed using hexane or chloroform, while moderately polar compounds like

apigenin and eupatilin partition into ethyl acetate. The ethyl acetate fraction is then concentrated and dried for further purification.

A simplified schematic for the extraction workflow is shown below:

Flowchart of conventional extraction process

1. Plant material selection and authentication
2. Drying and pulverization
3. Defatting with petroleum ether
4. Extraction (Maceration/Percolation/Soxhlet/Reflux)
5. Filtration and concentration
6. Solvent–solvent partitioning
7. Fractionation and drying

Evaluation of Extraction Efficiency

Extraction efficiency is evaluated based on extractive yield, flavonoid content, and purity. Spectrophotometric and chromatographic methods are used for quantification. Total flavonoid content can be estimated using aluminum chloride colorimetric assay, where apigenin equivalents are calculated. HPLC quantification provides accurate yield data, confirming extraction reproducibility.

Key Parameters Affecting Extraction Efficiency:

- Solvent polarity and concentration
- Temperature and duration
- Solvent-to-solid ratio (typically 10:1–20:1 mL/g)
- Particle size (40–60 mesh optimal)
- Stirring or agitation frequency

Advanced and Green Extraction Methods

Rapid advances in extraction science have replaced slow, solvent-intensive classical methods with energy-efficient, high-yield processes. These modern techniques maintain compound integrity, shorten extraction time, and minimize solvent waste, aligning with *green chemistry* principles. For apigenin and eupatilin—moderately lipophilic flavones—technologies such as **ultrasound-assisted extraction (UAE)**, **microwave-assisted extraction (MAE)**, **supercritical-fluid extraction (SFE)**, and **pressurized-liquid extraction (PLE)** have proven highly effective.

Ultrasound-Assisted Extraction (UAE)

UAE uses acoustic cavitation to disrupt cell walls, allowing solvent penetration and rapid solute diffusion. Cavitation bubbles collapse, generating local hotspots (~5000 K, 100 atm) that fragment cell membranes without significant heating of the bulk solvent.

Optimized Parameters for Apigenin

- Solvent: 70 % ethanol
- Frequency: 40 kHz
- Temperature: 50 °C
- Time: 30 min
- Solvent-to-solid ratio: 15:1 mL g⁻¹
- Yield $\approx$ 2.4 % w/w (1.5-fold increase over maceration)

Optimized Parameters for Eupatilin

- Solvent: 80 % methanol
- Frequency: 35 kHz
- Temperature: 55 °C
- Time: 25 min
- Yield $\approx$ 3.0 % w/w

UAE advantages include short duration, mild thermal load, and scalability via probe or bath systems. Limiting factors are solvent foaming and uneven energy distribution.

Microwave-Assisted Extraction (MAE)

MAE relies on dipole rotation and ionic conduction to heat the solvent-plant matrix volumetrically. Polar solvents absorb microwave energy efficiently, causing rapid cell rupture and solvent infiltration.

Process Parameters

- Solvent: 80 % ethanol for apigenin; 80 % acetone-ethanol for eupatilin
- Temperature: 110–130 °C
- Time: 5–10 min
- Microwave power: 600–800 W
- Pressure: 0.6 MPa

MAE yields 3.5–3.8 mg g⁻¹ of apigenin from chamomile and 3.8–4.0 mg g⁻¹ of eupatilin from *Artemisia asiatica*. Solvent use is reduced by 70 % compared with Soxhlet. Over-exposure causes oxidation; hence, closed-vessel systems with temperature feedback are preferred.

Supercritical-Fluid Extraction (SFE)

SFE employs supercritical CO₂ (critical point = 31 °C, 73 bar) as the solvent. Low viscosity and high diffusivity allow deep matrix penetration. Since apigenin and eupatilin are polar compared with CO₂, modifiers (“co-solvents”) such as ethanol enhance solubility.

Typical Conditions

- Solvent: CO₂ + 10 % ethanol
- Pressure: 35–45 MPa
- Temperature: 60 °C
- Flow rate: 3 mL min⁻¹
- Extraction time: 60 min

SFE produces 1.9 % apigenin and 1.7 % eupatilin yields. Major benefits are absence of residual solvents, short processing time, and tunable selectivity through pressure–temperature control.

Table: Comparative Performance of Modern Extraction Methods

Method	Solvent	Temp (°C)	Time	Pressure (MPa)	Yield (%) Apigenin	Yield (%) Eupatilin	Solvent Use (mL g ⁻¹)	Comments
UAE	70 % EtOH	50	0.5 h	–	2.4	3.0	10	Low energy, gentle
MAE	80 % EtOH	120	0.15 h	0.6	3.6	3.8	8	Fast, efficient
SFE	CO ₂ + EtOH	60	1 h	40	1.9	1.7	5	Clean extract
PLE	EtOH/H ₂ O (8:2)	100	0.25 h	10	2.7	3.1	9	High throughput

(Table: Performance comparison of advanced extraction methods for apigenin and eupatilin)

Pressurized-Liquid Extraction (PLE) / Accelerated Solvent Extraction

PLE uses elevated temperature and pressure to keep the solvent in liquid state above its boiling point, increasing solubility and diffusion. Parameters for flavones:

- Solvent: Ethanol-water (80:20)
- Temperature: 100 °C
- Pressure: 10 MPa
- Static cycle: 5 min × 3
- Flush volume: 60 %

- Yield $\approx$ 2.6–3.0 %

PLE offers reproducibility and automation, enabling sequential extractions with different solvents for polarity fractionation.

Optimization by Response-Surface Methodology (RSM)

RSM mathematically correlates extraction parameters with yield. Optimal apigenin extraction from *M. chamomilla* occurs at 74 % ethanol, 55 °C, 42 min; predicted yield = 2.54 %. For eupatilin, 80 % methanol, 60 °C, 30 min gives 3.82 mg g⁻¹. Regression R² > 0.98 confirms accuracy.

Key features:

- **Input:** Authenticated plant powder (40–60 mesh)
- **Extraction Chamber:** Ultrasonic bath or microwave reactor
- **Control Parameters:** Time, power, solvent ratio
- **Output:** Flavone-rich extract → filtration → concentration

Environmental and Economic Considerations

Green extraction minimizes hazardous solvent use, energy consumption, and waste generation. Life-cycle assessments show MAE and UAE reduce carbon footprint by $\approx$ 60 % compared with Soxhlet. CO₂ SFE systems have high capital cost but negligible solvent disposal expense, making them sustainable long-term.

Purification, Characterization, and Quality Control)

Fractionation and Isolation

Crude extracts are concentrated under reduced pressure ($\leq$ 45 °C) and suspended in distilled water for solvent–solvent partitioning:

1. **Defatting:** n-hexane or petroleum ether removes chlorophylls and waxes.
2. **Intermediate Fractionation:** Chloroform removes terpenoids.
3. **Target Fraction:** Ethyl acetate captures apigenin and eupatilin (moderate polarity).
4. **Residual Polar Fraction:** n-butanol retains glycosides and sugars.

The ethyl-acetate layer is concentrated and subjected to **open-column chromatography** on silica gel (60–120 mesh). A gradient elution of chloroform–ethyl acetate–methanol (100:0:0 → 0:90:10) separates sub-fractions. Each fraction is monitored by TLC under UV 365 nm.

TLC System Examples

- Apigenin R_f $\approx$ 0.42 (ethyl acetate:formic acid:water = 8:1:1)

- Eupatilin $R_f \approx 0.56$ (chloroform:methanol = 9:1)
- Visualization with $AlCl_3$ gives yellow fluorescence.

Final purification uses **Preparative HPLC**:

- Column: C18 (250 × 10 mm, 5 μ m)
- Mobile phase: Methanol–water (70:30 v/v) + 0.1 % formic acid
- Flow: 2 mL min⁻¹
- Detection: UV at 335 nm (apigenin), 340 nm (eupatilin)
- Purity > 98 %.

Spectroscopic Characterization

Table: Characteristic Spectral Data

Technique	Apigenin	Eupatilin	Interpretation
UV–Vis (nm)	268, 336	272, 340	Flavone band I (B-ring C=O), band II (A-ring OH)
IR (cm ⁻¹)	3400 (–OH), 1650 (C=O)	3420 (–OH), 1638 (C=O), 2850 (–OCH ₃)	Hydroxyl and methoxy identification
¹ H NMR (δ ppm)	6.1–7.9 (aromatic H)	3.80 (OCH ₃), 6.3–7.8 (aromatic H)	Aromatic pattern confirmation
MS (m/z)	270 [M ⁺]	344 [M ⁺]	Molecular ion peaks

(Table: Spectroscopic identification parameters for isolated flavones)

Quantification and Standardization

High-performance chromatographic techniques ensure batch-to-batch consistency.

HPLC Assay Parameters

- Column: C18 (250 × 4.6 mm)
- Mobile phase: Methanol–Water (60:40, v/v) + 0.1 % formic acid
- Flow rate: 1 mL min⁻¹
- Detection: UV 335 nm
- Calibration range: 1–100 μ g mL⁻¹ ($R^2 \geq 0.999$)

HPTLC Assay

- Stationary phase: Silica gel 60 F₂₅₄
- Mobile phase: Toluene:Ethyl acetate:Formic acid (6:3:1)

- Detection: Densitometric scan at 335 nm
- LOD: 20 ng spot⁻¹

Standardization data guide pharmacopoeial monograph preparation for *Matricaria chamomilla* and *Artemisia asiatica* extracts.

Stability and Storage

Apigenin and eupatilin degrade in alkaline or high-temperature environments via oxidative cleavage of the C-ring. Stability studies show < 5 % loss over 6 months at 4 °C in amber vials, but > 20 % loss at 40 °C/75 % RH. Protective strategies include:

- Encapsulation in β -cyclodextrin inclusion complexes
- Antioxidant addition (ascorbic acid 0.1 %)
- Nitrogen flushing during storage

Quality-Control Flow and Description

(Suggested Schematic) Displays Analytical Workflow:

1. Extraction → 2. Fractionation → 3. Chromatography → 4. Spectroscopic Characterization → 5. Quantification → 6. Stability Testing.
2. Each stage links to validated protocols (HPLC/HPTLC/IR/NMR/MS) ensuring traceability and reproducibility.

Integration with Industrial and Pharmacognostic Standards

The World Health Organization (WHO) and European Pharmacopoeia recommend standardized markers for herbal materials. Apigenin content in chamomile should not fall below 0.5 % w/w, while eupatilin in *Artemisia asiatica* extracts must exceed 0.3 % w/w. Extracts meeting these thresholds are considered pharmacologically active for anti-inflammatory or gastroprotective use.

Table: Pharmacognostic Standards for Apigenin- and Eupatilin-Rich Extracts

Parameter	Chamomile Extract	<i>Artemisia asiatica</i> Extract	Analytical Method
Foreign Matter (%)	≤ 2.0	≤ 2.0	Microscopic examination
Moisture Content (%)	≤ 10	≤ 9	Loss on drying
Total Ash (%)	≤ 7	≤ 8	Incineration

Parameter	Chamomile Extract	<i>Artemisia asiatica</i> Extract	Analytical Method
Extractive Value (% 70 % EtOH)	≥ 20	≥ 18	Gravimetric
Apigenin/Eupatilin Assay (% w/w)	≥ 0.5	≥ 0.3	HPLC/HPTLC
Heavy Metals (ppm)	Pb < 10 ; As < 3	Pb < 10 ; As < 3	AAS Method

Isolation, Purification, and Characterization

Post-Extraction Handling

Once crude extract is collected, solvent is evaporated under reduced pressure below 45 °C using a rotary evaporator to preserve thermolabile flavones. The concentrated residue is weighed to calculate extractive yield, then re-dissolved in minimal ethanol or methanol for fractionation. Residual water is removed by lyophilization, producing a dry extract stable for ≥ 12 months at 4 °C in amber glass.

Fractionation Workflow

Table: Solvent–Solvent Partition Scheme

Step	Solvent Used	Purpose	Expected Fraction
1	n-Hexane	Removes lipids, chlorophyll, wax	Defatted residue
2	Chloroform	Removes terpenoids, pigments	Intermediate fraction
3	Ethyl acetate	Extracts flavones (apigenin, eupatilin)	Target fraction
4	n-Butanol	Extracts glycosides, phenolics	Polar residue
5	Water	Removes inorganic salts	Final wash

Ethyl-acetate fraction contains the aglycones apigenin and eupatilin, both sparingly soluble in water ($\log P \approx 2.5\text{--}3.0$).

Column Chromatography

Silica gel (60–120 mesh) is the stationary phase of choice.

- **Column size:** 4 × 60 cm
- **Sample loading:** 2 g extract / 10 g silica
- **Eluent gradient:** Chloroform → ethyl acetate → methanol (100:0:0 → 0:80:20)

- **Flow rate:** 2 mL min⁻¹

Fractions (10 mL each) are monitored by TLC. Fractions showing identical R_f values are pooled and concentrated.

Table: TLC solvent systems

Compound	Mobile Phase	R _f Value	Detection
Apigenin	Ethyl acetate:formic acid:water (8:1:1)	0.42	UV 365 nm, yellow fluorescence with AlCl ₃
Eupatilin	Chloroform:methanol (9:1)	0.56	UV 340 nm, greenish-yellow spot

Preparative and Flash Chromatography

For higher throughput, flash systems using pre-packed silica or C18 cartridges are employed.

Example setup

- Column: C18 (250 × 21 mm)
- Mobile phase: Methanol–water (70:30, v/v) with 0.1 % formic acid
- Flow: 10 mL min⁻¹
- Detector: UV 335 nm
- Fraction collector programmed every 1 min

Apigenin elutes at t_R = 13.8 min; eupatilin at t_R = 16.1 min. Purity > 98 % confirmed by analytical HPLC.

Crystallization

Pure fractions are evaporated, then recrystallized from hot ethanol.

- **Apigenin:** Pale-yellow needles, mp ≈ 347 °C
- **Eupatilin:** Yellow-brown prisms, mp ≈ 320 °C
- Crystallization confirms purity visually and by melting-point uniformity (± 1 °C deviation).

Spectroscopic Identification

Table: Spectral Characteristics of Isolated Flavones

Technique	Apigenin (Data)	Eupatilin (Data)	Diagnostic Interpretation
UV–Vis (nm)	268, 336	272, 340	Band I (B-ring C=O) and Band II (A-ring –OH)

Technique	Apigenin (Data)	Eupatilin (Data)	Diagnostic Interpretation
IR (cm ⁻¹)	3400 (–OH), 1650 (C=O), 1610 (C=C)	3420 (–OH), 1638 (C=O), 2850 (–OCH ₃)	Hydroxyl and methoxy groups
¹ H NMR (δ ppm)	6.1 (1H, d), 6.5 (1H, s), 7.9 (2H, d)	3.80 (3H, s, OCH ₃), 6.4–7.8 (ArH)	Aromatic pattern confirmation
¹³ C NMR (δ ppm)	182 (C=O), 164–100 (Ar C)	180 (C=O), 160–98 (Ar C), 56 (OCH ₃ C)	Flavone skeleton
MS (m/z)	270 [M ⁺]	344 [M ⁺]	Molecular ion peaks
HPLC tR (min)	13.8	16.1	Distinct peaks at 335 nm

(Table: summarizes key analytical fingerprints.)

Quantification and Calibration

HPLC is the standard quantification method.

System parameters

- Column: C18 (250 × 4.6 mm, 5 μm)
- Mobile phase: Methanol–Water (60:40) + 0.1 % formic acid
- Flow: 1 mL min⁻¹
- Detection: 335 nm
- Injection volume: 20 μL
- Calibration range: 0.5–100 μg mL⁻¹ (R² ≥ 0.999)

Table: Validation summary

Parameter	Apigenin	Eupatilin
Linearity (μg mL ⁻¹)	0.5–100	0.5–100
LOD (μg mL ⁻¹)	0.05	0.07
LOQ (μg mL ⁻¹)	0.15	0.20
Precision (RSD %)	≤ 2	≤ 2
Recovery (%)	99.2	98.7

Densitometric HPTLC methods show similar accuracy, suitable for routine quality control.

Table: Quality-Control Parameters

Parameter	Acceptable Range	Analytical Method
Moisture (%)	≤ 10	Loss on Drying
Total Ash (%)	≤ 8	Incineration
Acid-Insoluble Ash (%)	≤ 2	Gravimetric
Extractive Value (% EtOH)	≥ 18	Gravimetric
Heavy Metals (ppm)	Pb < 10, As < 3	AAS
Microbial Load (CFU g ⁻¹)	< 10 ³	Plate Count
Marker Compound Content (% w/w)	Apigenin ≥ 0.5 , Eupatilin ≥ 0.3	HPLC Assay

Stability Studies

Accelerated testing (40 °C $\pm$ 2 °C, 75 % RH $\pm$ 5 %) for 6 months shows:

- Apigenin loss $\approx$ 18 %
- Eupatilin loss $\approx$ 22 %

At 4 °C, degradation < 5 %. Decomposition follows first-order kinetics. Shelf-life (t_{90}) $\approx$ 18 months.

Protection strategies:

- Store under nitrogen atmosphere.
- Add 0.1 % ascorbic acid.
- Use amber vials and light-proof packaging.

Analytical Workflow

1. Plant authentication $\rightarrow$ 2. Drying & milling $\rightarrow$ 3. Optimized extraction (UAE/MAE/SFE) $\rightarrow$ 4. Fractionation $\rightarrow$ 5. Chromatographic purification $\rightarrow$ 6. Spectroscopic characterization $\rightarrow$ 7. HPLC quantification $\rightarrow$ 8. QC validation.

Each step aligns with *ICH Q2 (R1)* and *WHO Herbal Monograph* guidelines.

Industrial Integration

Large-scale recovery employs counter-current extraction or simulated moving-bed chromatography.

- Solvent recovery via distillation achieves > 95 % recycling.
- Continuous UAE reactors handle > 50 kg biomass per batch.
- Supercritical CO₂ units process up to 200 L CO₂ h⁻¹ for eco-friendly operation.

Economic data indicate production cost $\approx$ US\$ 70 kg⁻¹ pure apigenin and US\$ 95 kg⁻¹ eupatilin when solvent recycling and heat recovery are optimized.

Pharmacognostic Significance

Apigenin and eupatilin serve as diagnostic chemical markers for their respective plants.

- TLC/HPTLC fingerprints ensure botanical identity.
- HPLC retention times and UV spectra confirm authenticity.
- Presence or absence of specific peaks distinguishes adulterants or substitutes.

Such chromatographic profiles are essential for pharmacognostic monograph development of *Matricaria chamomilla*, *Artemisia asiatica*, and related species.

Correlation Between Extraction Method and Bioactivity

Studies reveal a positive correlation between extraction efficiency and biological potency. UAE and MAE extracts, containing higher flavone concentration, exhibit stronger anti-inflammatory and antioxidant effects than macerated extracts.

Extraction Type	Apigenin Yield (%)	DPPH Inhibition (%)	COX-2 Inhibition (IC ₅₀ μ M)
Soxhlet	1.2	60 $\pm$ 2	28.4
UAE	2.4	78 $\pm$ 1	15.2
MAE	3.6	82 $\pm$ 1	12.7

(Table: shows higher yield $\rightarrow$ greater bioactivity correlation.)

Regulatory Aspects

WHO, EMA, and US-FDA botanical guidelines require validated analytical markers and solvent limits. Residual solvent thresholds for ethanol/methanol extracts: < 0.3 %. CO₂ extraction classified as “Class III” solvent process, requiring no residue testing.

Environmental and Safety Considerations

- Solvent recycling reduces environmental load by > 80 %.

- Ethanol preferred over methanol for nontoxicity.
- Supercritical CO₂ systems eliminate hazardous waste.
- Spent plant material serves as bio-fertilizer after composting.

Pharmacological Activities of Apigenin

Antioxidant activity.

Apigenin functions as both a direct radical scavenger and an indirect inducer of endogenous antioxidant defenses. Its three free hydroxyl groups permit hydrogen donation to neutralize reactive oxygen and nitrogen species, and its planar flavone scaffold stabilizes resulting radicals. More importantly, apigenin activates the Nrf2/ARE pathway: it reduces Keap1-mediated Nrf2 degradation, fosters Nrf2 nuclear translocation, and increases transcription of HO-1, NQO1, GCL and other phase-II/detoxifying enzymes, raising intracellular GSH and antioxidant enzyme activities (SOD, catalase). Apigenin also reduces ROS production by inhibiting enzymes such as NADPH oxidase and xanthine oxidase. These dual actions (direct scavenging + Nrf2 induction) have been shown to reduce lipid peroxidation (MDA/TBARS), DNA oxidation, and mitochondrial dysfunction in hepatocytes, neurons and cardiomyocytes across multiple in vitro and in vivo studies.

Anti-inflammatory and analgesic effects.

Apigenin suppresses innate and adaptive inflammatory signaling primarily by inhibiting NF- κ B and by modulating MAPKs. Mechanistically, apigenin prevents I κ B α phosphorylation and degradation, thereby blocking NF- κ B (p65) nuclear translocation and downstream transcription of iNOS, COX-2, TNF- α , IL-1 β and IL-6. It also attenuates phosphorylation of p38, JNK and ERK, which further lowers cytokine and prostaglandin production. At the effector level this reduces NO and PGE₂ release, leukocyte recruitment, and vascular adhesion molecule expression. Analgesic effects in formalin, hot-plate and writhing tests parallel these anti-inflammatory mechanisms and may include modulation of peripheral nociceptor sensitization and central GABAergic tone. Multiple cell and animal studies report dose-dependent reduction in edema, cytokine levels and pain behaviors after apigenin treatment.

Anticancer potential.

Apigenin is a multitargeted chemopreventive and antitumor agent. It induces cell-cycle arrest (G₀/G₁ or G₂/M depending on cell type) via downregulation of cyclins and CDKs and

upregulation of CDK inhibitors p21/p27. Apigenin triggers apoptosis by shifting the Bax/Bcl-2 balance, promoting mitochondrial outer membrane permeabilization, cytochrome c release, caspase-9/-3 activation and PARP cleavage. It suppresses pro-survival signaling (PI3K/Akt, STAT3), inhibits MAPK/ERK in some contexts, and interferes with oncogenic transcription factors. Anti-angiogenic effects arise from reduced HIF-1 α and VEGF expression and impaired endothelial migration/tube formation, while anti-metastatic activity stems from downregulation of MMP-2/-9 and uPA. Apigenin also modulates epigenetic marks and microRNA networks in cancer cells and can sensitize chemoresistant tumors to conventional drugs (cisplatin, 5-FU, doxorubicin) by blocking survival pathways and transporters. Systematic reviews and numerous xenograft studies confirm tumor growth inhibition with limited toxicity to normal tissue.

Neuroprotective and cognitive effects.

Apigenin crosses the blood–brain barrier and protects neural tissue through antioxidant, anti-inflammatory and anti-apoptotic mechanisms. It activates neuronal Nrf2/HO-1 signaling, reduces microglial activation via NF- κ B and MAPK inhibition, and limits nitric oxide and proinflammatory cytokines in the CNS. Apigenin reduces amyloid- β production by inhibiting BACE1 and lowers tau hyperphosphorylation through GSK-3 β modulation; both actions translate into reduced plaque/tangle pathology in Alzheimer's models. It also enhances neurotrophic signaling (BDNF/CREB) and modulates GABA_A receptor benzodiazepine sites, producing anxiolytic effects with minimal sedation. Preclinical stroke, traumatic brain injury and transgenic Alzheimer's studies report reduced infarct size, preserved synaptic function and improved learning/memory after apigenin administration.

Cardioprotective actions.

Apigenin protects the heart by multiple complementary mechanisms. In ischemia–reperfusion and myocardial infarction models it decreases infarct size, lowers apoptotic cardiomyocytes, reduces plasma CK-MB/LDH release and preserves contractile function. Molecularly, apigenin limits oxidative stress, stabilizes mitochondria, upregulates anti-apoptotic Bcl-2 and downregulates Bax and caspase-3 activation. It improves endothelial function by enhancing eNOS activity and NO bioavailability, reduces vascular inflammation by suppressing adhesion molecules and cytokines, and inhibits LDL oxidation and foam cell formation—actions that slow atherogenesis. Several systematic reviews and experimental reports document cardioprotective efficacy and improved post-ischemic recovery with apigenin.

Antidiabetic and metabolic effects.

Apigenin exerts insulin-sensitizing, glucose-lowering and lipid-modulating effects. It activates AMPK in liver, muscle and adipose tissue, promoting GLUT4 translocation and glucose uptake. Apigenin improves insulin signaling via enhanced IRS phosphorylation and reduces hepatic gluconeogenesis. Its antioxidant and anti-inflammatory actions protect pancreatic β -cells from cytokine and oxidative damage, preserving insulin secretion. Apigenin also downregulates SREBP-1c and lipogenic genes while upregulating PPAR α -mediated fatty acid oxidation, improving steatosis and lipid profiles in high-fat diet and diabetic models. Experimental data show lower fasting glucose, better glucose tolerance and improved lipid parameters after apigenin supplementation.

Antimicrobial and antiviral effects.

Apigenin demonstrates broad antimicrobial activity against Gram-positive and Gram-negative bacteria and antifungal activity against *Candida* species. Mechanisms include inhibition of bacterial enzymes (e.g., DNA gyrase), disruption of membrane integrity at higher concentrations, interference with quorum sensing and biofilm formation, and synergy with antibiotics against resistant strains. Antiviral actions have been described against influenza, HSV and in vitro reports for coronaviruses, where apigenin impairs viral proteases, polymerases or host factors essential for replication. These activities are largely preclinical but suggest adjuvant potential.

Hepatoprotective and anti-fibrotic actions.

Liver protection by apigenin follows from its antioxidant, anti-inflammatory and anti-TGF- β actions. Apigenin attenuates chemically induced hepatotoxicity (CCl₄, paracetamol) by restoring hepatic antioxidant enzymes and reducing inflammatory cytokines, transaminases and necrosis. In fibrosis models, it inhibits TGF- β /Smad signaling, decreases fibroblast activation and collagen deposition, and improves histological architecture, supporting potential for chronic liver disease adjunct therapy.

Bone and musculoskeletal effects.

Apigenin promotes osteoblast differentiation and mineralization while inhibiting osteoclastogenesis. Mechanistically it stimulates Runx2 and Wnt/ β -catenin signaling in osteoblasts, increases expression of osteocalcin and alkaline phosphatase, and suppresses RANKL-induced NF- κ B activation in osteoclast precursors. Animal studies show improved bone

density and reduced resorption in osteoporotic models, suggesting translational potential for osteoporosis.

Nephroprotective Effects

In cisplatin- and gentamicin-induced nephrotoxicity, apigenin reduced creatinine, BUN, and MDA levels while upregulating HO-1 and Nrf2. It prevents tubular apoptosis by inhibiting p53 and caspase-3 (Kumar et al., *Toxicology Reports*, 2019).

Gastroprotective and Antiulcer Activity

Apigenin protects gastric mucosa by enhancing mucin secretion, reducing acid output, and inhibiting H^+/K^+ -ATPase. It downregulates inflammatory mediators like $TNF-\alpha$ and $IL-1\beta$ in gastric tissues. In ethanol-induced ulcer models, apigenin reduced ulcer index and restored mucosal integrity (Sharma et al., *Journal of Ethnopharmacology*, 2018).

Anti-obesity and Lipid-lowering Effects

Apigenin inhibits adipocyte differentiation by downregulating $PPAR\gamma$, $C/EBP\alpha$, and $SREBP-1c$. It increases lipolysis and fatty acid oxidation through AMPK activation. In high-fat diet mice, it reduced body weight gain and serum triglycerides (Xu et al., *Nutrients*, 2020).

Anti-aging and Longevity Effects

Apigenin increases lifespan and stress resistance by activating SIRT1 and AMPK, which enhance mitochondrial function and reduce oxidative DNA damage. In *Caenorhabditis elegans*, apigenin extended lifespan by 20% (Park et al., *Mechanisms of Ageing and Development*, 2019).

Reproductive Health Effects

Apigenin improves sperm count and motility, protects against testicular oxidative stress, and regulates hormone balance. It also exhibits mild phytoestrogenic activity via weak binding to $ER\beta$, potentially useful for menopausal symptoms (Nakamura et al., *Reproductive Toxicology*, 2020).

Immunomodulatory Effects

Apigenin suppresses overactive immune responses while promoting regulatory T-cell function. It reduces Th1 and Th17 cytokines and upregulates IL-10. In autoimmune models, it alleviated symptoms by inhibiting $NF-\kappa B$ and STAT1 signaling (Sultan et al., *Immunopharmacology and Immunotoxicology*, 2021).

Dermatological and Wound-healing Effects

Apigenin promotes collagen synthesis, fibroblast proliferation, and angiogenesis, accelerating wound closure. It reduces UV-induced ROS, MMP-1, and pigmentation by suppressing tyrosinase and MITF expression. Topical formulations (0.1–1%) improved healing rates in rat and human skin models (Sharma et al., Skin Pharmacology and Physiology, 2022).

Pulmonary Protective and Antiasthmatic Effects

Apigenin inhibits airway inflammation by reducing IL-4, IL-5, and eosinophil infiltration. It modulates Th2 cytokine balance and relaxes airway smooth muscles by blocking Ca^{2+} influx. In OVA-induced asthma models, apigenin improved airway resistance and lung histology (Li et al., Pulmonary Pharmacology & Therapeutics, 2019).

Antiviral and Antiparasitic Potential

Beyond influenza and HSV, apigenin inhibits Hepatitis C virus replication by targeting NS5B polymerase, and *Plasmodium falciparum* by interfering with heme detoxification. These mechanisms highlight broad antiviral and antiparasitic actions needing further clinical validation.

Antifibrotic and Antiproliferative Vascular Effects

Apigenin reduces vascular smooth muscle cell (VSMC) proliferation by blocking ERK1/2 and Akt signaling, thus preventing neointima formation after vascular injury (Zhou et al., Atherosclerosis, 2018).

Dermatological and cosmetic properties.

Topically, apigenin protects skin from UV-induced oxidative damage and inflammation, inhibits melanogenesis (tyrosinase suppression and modulation of MAPKs), and improves barrier function—properties that support anti-aging and depigmenting applications in cosmetic formulations. In vivo skin models and ex vivo human skin studies show reduced erythema and oxidative markers after apigenin application.

Pharmacokinetics, bioavailability and formulation approaches.

A major translational barrier is apigenin's poor aqueous solubility and low oral bioavailability due to limited absorption and extensive phase-II metabolism (glucuronidation/sulfation). Human ADME studies and reviews report low systemic levels of unmetabolized apigenin after oral intake and rapid formation of conjugated metabolites. To address this, numerous formulation strategies have been developed—cyclodextrin inclusion complexes, lipid nanoparticles, solid

lipid carriers, phytosomes, and polymeric nanocarriers—that enhance solubility, protect against presystemic metabolism, and improve tissue delivery. Several recent preclinical studies demonstrate markedly increased plasma exposure and improved pharmacodynamic effects with nanoformulations. Human clinical data remain sparse but emerging trials on safety, absorption and small therapeutic endpoints have been reported.

Safety, drug interactions and translational considerations.

Apigenin shows a favorable safety profile in acute and subchronic animal studies, with high LD₅₀ values and little organ toxicity at pharmacologically active doses. However, apigenin modulates drug-metabolizing enzymes (notably UGTs and CYPs) and transporters in vitro, raising the possibility of pharmacokinetic interactions when coadministered with substrates of these pathways; this requires attention in clinical development. Standardization of extracts, validated marker assays, and formulation optimization are critical steps to translate robust preclinical efficacy into clinical trials. Systematic and well-designed human studies remain the primary gap.

Representative literature summary (select recent reviews and experimental studies).

Recent comprehensive reviews synthesize apigenin’s multi-system actions, mechanisms and translational hurdles (Salehi et al., 2019; Chen et al., 2023; Xu et al., 2022), while focused systematic analyses cover anticancer (Singh et al., 2022; Daneshvar et al., 2023) and cardiometabolic effects. Key experimental studies include cardioprotection in ischemia–reperfusion models (Hu et al., 2015), neuroprotection targeting GSK-3β and BACE1 (Alsadat et al., 2021; Zhang et al., 2024), and multiple ADME/bioavailability assessments highlighting metabolic conjugation and low oral exposure (Wang et al., 2019; Borges et al., 2022). These works collectively document the mechanistic rationale and preclinical efficacy while identifying bioavailability and clinical evidence as priorities for future research.

Summary Table: Major Mechanisms and Effects of Apigenin

Pharmacological Activity	Major Mechanism	Key Targets/Pathways	Reference Example
Antioxidant	Nrf2 activation, radical scavenging	HO-1, GPx, SOD	Shukla & Gupta, 2010
Anti-inflammatory	NF-κB and MAPK inhibition	COX-2, TNF-α, IL-6	Nicholas et al., 2021
Anticancer	Apoptosis, epigenetic regulation	PI3K/Akt, p53, Bcl-2	Yao et al., 2022
Neuroprotective	BDNF, GABA modulation	GSK-3β, BACE1	Zhao et al., 2021

Pharmacological Activity	Major Mechanism	Key Targets/Pathways	Reference Example
Cardioprotective	eNOS, antioxidant defense	PI3K/Akt, NF- κ B	Hu et al., 2015
Antidiabetic	AMPK activation	GLUT4, PEPCK	Kim et al., 2020
Hepatoprotective	TGF- β /Smad inhibition	GSH, MDA	Li et al., 2018
Gastroprotective	H ⁺ /K ⁺ -ATPase inhibition	TNF- α , IL-1 β	Sharma et al., 2018
Antimicrobial	DNA gyrase inhibition	Bacteria & fungi	Zhang et al., 2021

Pharmacological Activities of Eupatilin

Anti-ulcer and Gastroprotective Activity

Eupatilin exhibits potent anti-ulcer and gastroprotective properties supported by extensive preclinical and clinical data. Its primary action involves suppression of gastric mucosal inflammation, inhibition of oxidative damage, and reinforcement of mucosal defense mechanisms. Eupatilin scavenges reactive oxygen species (ROS) and upregulates endogenous antioxidants, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). In ethanol- and indomethacin-induced ulcer models, eupatilin significantly reduces gastric lesion indices, hemorrhage, and mucosal erosion. The mechanism includes inhibition of neutrophil infiltration, suppression of myeloperoxidase activity, and downregulation of COX-2 and iNOS expression.

At the cellular level, eupatilin stabilizes gastric epithelial cell membranes and maintains mitochondrial integrity. Studies in *Artemisia asiatica* extract demonstrated that eupatilin suppresses NF- κ B nuclear translocation and reduces secretion of TNF- α , IL-1 β , and IL-6. It also stimulates prostaglandin E₂ (PGE₂) synthesis, enhancing mucosal blood flow and epithelial regeneration. Jeong et al. (2009) reported that eupatilin pretreatment attenuated ethanol-induced mucosal apoptosis by increasing Bcl-2 expression and inhibiting Bax-mediated mitochondrial permeability transition.

In NSAID-induced gastropathy, eupatilin prevents gastric mucosal necrosis by maintaining tight junction integrity through modulation of occludin and claudin-1. Clinical formulations such as Stillen® (DA-9601), containing standardized eupatilin and jaceosidin fractions from *Artemisia asiatica*, show significant symptom relief in chronic gastritis and gastric ulcer patients without major adverse effects. Therefore, eupatilin represents one of the few plant-derived flavones with well-characterized gastroprotective mechanisms and translational efficacy.

Anti-inflammatory and Antioxidant Properties

Eupatilin acts as a dual anti-inflammatory and antioxidant molecule, targeting both enzymatic and transcriptional pathways. Its anti-inflammatory action arises from the inhibition of NF- κ B, AP-1, and MAPK signaling cascades. In macrophages stimulated with lipopolysaccharide (LPS), eupatilin suppresses phosphorylation of I κ B α , p38 MAPK, ERK1/2, and JNK, resulting in decreased transcription of iNOS, COX-2, and pro-inflammatory cytokines such as IL-6 and TNF- α .

The antioxidant profile of eupatilin is enhanced by the presence of methoxy substituents at the 3',4',6 positions, which confer lipid solubility and membrane permeability. This facilitates its accumulation within cellular membranes where lipid peroxidation occurs. Eupatilin upregulates nuclear factor erythroid 2-related factor 2 (Nrf2), promoting the expression of phase II detoxifying enzymes including HO-1, NQO1, and glutathione-S-transferase (GST). These enzymes mitigate oxidative stress and restore cellular redox balance.

Ahn et al. (2015) demonstrated that eupatilin protects hepatic and neuronal cells from hydrogen peroxide-induced damage by increasing GSH levels and reducing ROS accumulation. In animal models, oral administration of eupatilin significantly decreases malondialdehyde (MDA) levels and increases antioxidant enzyme activity. It also prevents inflammatory edema and vascular permeability in carrageenan-induced paw edema and formalin-induced inflammation models. Therefore, eupatilin functions as a multi-target antioxidant and anti-inflammatory flavone capable of modulating upstream signal transduction, enzymatic antioxidant systems, and gene expression networks.

Anticancer and Chemopreventive Roles

Eupatilin exhibits broad-spectrum anticancer properties across multiple cancer cell lines, including gastric, lung, liver, breast, and colorectal cancers. Its mechanisms of action involve induction of apoptosis, inhibition of cell proliferation, suppression of angiogenesis, and interference with epithelial-mesenchymal transition (EMT).

In gastric cancer cell lines (AGS, MKN-45), eupatilin induces apoptosis through activation of caspase-3 and -9, mitochondrial cytochrome c release, and downregulation of Bcl-2. It also inhibits PI3K/Akt and STAT3 signaling, leading to cell cycle arrest at the G2/M phase. In lung carcinoma (A549), eupatilin suppresses cancer cell motility and invasion by decreasing MMP-2 and MMP-9 expression, while upregulating E-cadherin, indicating reversal of EMT.

Eupatilin's chemopreventive role extends to modulation of oxidative DNA damage and inhibition of carcinogen-activating enzymes. It downregulates CYP1A1 and CYP2E1, reducing formation of reactive carcinogenic metabolites. In colon carcinogenesis models induced by azoxymethane, eupatilin treatment reduced aberrant crypt foci and inflammatory cell infiltration in colonic mucosa.

Additionally, eupatilin inhibits angiogenesis by blocking VEGF-induced endothelial cell proliferation and tube formation through suppression of VEGFR2 phosphorylation. In hepatocellular carcinoma models, eupatilin inhibits TGF- β 1-induced EMT by preventing Smad2/3 activation, thereby impeding metastatic potential.

Synergistic studies reveal that eupatilin enhances the efficacy of conventional chemotherapeutic agents like cisplatin and doxorubicin while mitigating their oxidative side effects. This suggests its utility as an adjuvant phytochemical in integrative cancer therapy.

Neuroprotective and Hepatoprotective Effects

Eupatilin shows strong neuroprotective and hepatoprotective activity, primarily through its antioxidant and anti-inflammatory mechanisms, as well as modulation of intracellular signaling pathways related to cell survival and apoptosis.

In neuronal models, eupatilin protects against β -amyloid (A β)-induced toxicity, oxidative stress, and glutamate excitotoxicity. It activates the Nrf2/HO-1 axis, suppresses microglial activation, and downregulates proinflammatory mediators in astrocytes. Park et al. (2014) reported that eupatilin prevents A β -induced neuronal apoptosis via inhibition of GSK-3 β and restoration of mitochondrial membrane potential. Eupatilin also enhances synaptic protein expression and cognitive performance in rodent models of Alzheimer's disease, suggesting potential therapeutic benefits for neurodegenerative conditions.

In hepatic systems, eupatilin exerts hepatoprotective effects against carbon tetrachloride (CCl₄), acetaminophen, and alcohol-induced liver injury. It reduces serum ALT, AST, and bilirubin levels while enhancing hepatic antioxidant enzyme activity. Eupatilin suppresses TGF- β 1-induced hepatic stellate cell activation, inhibiting collagen deposition and fibrosis progression. Its anti-fibrotic mechanism involves inhibition of Smad2/3 phosphorylation and restoration of Smad7 expression, which acts as a negative feedback regulator.

In non-alcoholic fatty liver disease (NAFLD) models, eupatilin improves lipid metabolism by activating AMPK and PPAR α , leading to decreased triglyceride accumulation

and hepatic steatosis. Collectively, these studies demonstrate that eupatilin's neuroprotective and hepatoprotective effects are mediated through integrated antioxidant, anti-inflammatory, and metabolic regulatory mechanisms.

Immunomodulatory Activity

Eupatilin displays dual immunomodulatory effects—suppressing excessive immune activation while maintaining host defense. In macrophages, it inhibits LPS-induced activation of Toll-like receptor 4 (TLR4) signaling, reducing the release of TNF- α , IL-6, and IL-1 β by blocking MyD88-dependent NF- κ B activation. This helps regulate innate immune overactivation in chronic inflammatory conditions.

In adaptive immunity, eupatilin suppresses Th1 and Th17 differentiation while promoting regulatory T cell (Treg) activity by enhancing FoxP3 expression and IL-10 secretion. These effects have been confirmed in experimental autoimmune encephalomyelitis (EAE) and rheumatoid arthritis models, where eupatilin reduced joint swelling and demyelination severity.

In mast cells and eosinophils, eupatilin inhibits Fc ϵ RI-mediated degranulation and histamine release, suggesting potential use in allergic and autoimmune diseases. It also modulates macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 phenotype, which is crucial in tissue repair and fibrosis prevention.

Anti-fibrotic and Tissue Remodeling Activity

Eupatilin strongly inhibits tissue fibrosis in liver, lung, and kidney models. Its primary mechanism involves attenuation of TGF- β 1/Smad signaling and suppression of extracellular matrix (ECM) deposition. In hepatic stellate cells, eupatilin reduces α -SMA, collagen type I, and fibronectin expression. Similar results were found in bleomycin-induced pulmonary fibrosis, where eupatilin prevented fibroblast proliferation and EMT progression.

Eupatilin also blocks non-Smad profibrotic pathways, including p38 MAPK, ERK, and PI3K/Akt. By upregulating matrix metalloproteinases (MMP-1 and MMP-9), it promotes ECM degradation and tissue remodeling. These combined effects position eupatilin as a potential therapeutic for fibrotic diseases such as idiopathic pulmonary fibrosis and liver cirrhosis.

Antidiabetic and Metabolic Regulation

Eupatilin enhances insulin sensitivity and glucose utilization by activating AMP-activated protein kinase (AMPK) in adipose tissue, liver, and muscle. This leads to increased glucose

uptake via GLUT4 translocation and reduced gluconeogenesis through inhibition of PEPCK and G6Pase expression.

In pancreatic β -cells, eupatilin protects against oxidative and cytokine-induced apoptosis by activating the PI3K/Akt pathway and suppressing caspase-3 activity. Animal studies demonstrate improved glucose tolerance and reduced fasting glucose in high-fat diet-induced diabetic mice after eupatilin treatment.

Furthermore, eupatilin modulates lipid metabolism by downregulating SREBP-1c and ACC, reducing triglyceride synthesis, and enhancing fatty acid oxidation via PPAR α upregulation. These effects make it beneficial for metabolic syndrome and non-alcoholic fatty liver disease (NAFLD).

Cardioprotective and Vascular Actions

Eupatilin exerts cardioprotective effects by maintaining endothelial integrity and reducing oxidative stress during ischemia–reperfusion injury. It activates eNOS, enhancing nitric oxide (NO) production, which promotes vasodilation and reduces vascular resistance.

In myocardial infarction models, eupatilin suppresses oxidative stress markers (MDA, ROS) and increases antioxidant enzymes (SOD, CAT). It inhibits cardiac hypertrophy by suppressing the MAPK and calcineurin–NFAT pathways. In vascular smooth muscle cells, eupatilin reduces Ca²⁺ influx and inhibits phenylephrine-induced contraction, contributing to antihypertensive potential.

It also prevents endothelial dysfunction by inhibiting ICAM-1 and VCAM-1 expression, reducing leukocyte adhesion and atherogenesis. These findings suggest eupatilin's potential in preventing hypertension, atherosclerosis, and ischemic heart diseases.

Anti-allergic and Anti-asthmatic Activity

Eupatilin suppresses allergic airway inflammation by inhibiting Th2 cytokines (IL-4, IL-5, IL-13) and IgE synthesis. In ovalbumin-induced asthma models, it reduces airway hyperresponsiveness, mucus production, and eosinophilic infiltration.

Mechanistically, eupatilin inhibits mast cell degranulation and histamine release via suppression of Lyn/Syk kinase signaling. It also stabilizes mast cell membranes, preventing excessive mediator release. In addition, eupatilin inhibits 5-lipoxygenase (5-LOX) and decreases leukotriene production, which mediates bronchoconstriction and airway remodeling.

These combined effects suggest eupatilin may serve as a complementary therapy for allergic rhinitis, asthma, and atopic dermatitis.

Dermatological and Wound-Healing Activity

Eupatilin promotes skin regeneration and wound healing through enhancement of keratinocyte migration and fibroblast proliferation. It accelerates re-epithelialization and collagen deposition while reducing inflammatory infiltration at wound sites.

In UVB-induced photoaging models, eupatilin decreases MMP-1 and MMP-3 expression, thereby preventing collagen breakdown and wrinkle formation. Its antioxidant action protects dermal fibroblasts from UV-induced oxidative stress, while increasing procollagen type I synthesis. Eupatilin also inhibits melanogenesis by downregulating tyrosinase and MITF expression, suggesting skin-lightening potential. In acne models, eupatilin reduces sebocyte lipid production and inflammatory cytokines, making it relevant for dermatological formulations.

Antimicrobial and Antiviral Activities

Eupatilin shows moderate antimicrobial activity against *Helicobacter pylori*, *Staphylococcus aureus*, and *Escherichia coli* by damaging bacterial membranes and inhibiting DNA gyrase and topoisomerase IV. Against *H. pylori*, eupatilin not only inhibits growth but also suppresses urease activity, reducing bacterial survival in acidic environments.

Antifungal activity against *Candida albicans* and *Aspergillus niger* has also been documented, possibly due to membrane lipid disruption. In viral models, eupatilin inhibits influenza virus replication by downregulating viral RNA polymerase activity and modulating host interferon response.

It also shows antiviral effects against hepatitis B virus (HBV) and hepatitis C virus (HCV) by reducing viral DNA replication and inhibiting HBsAg secretion. These findings highlight eupatilin's potential as a complementary antimicrobial and antiviral agent.

Analgesic and Antipyretic Activity

Eupatilin exhibits central and peripheral analgesic effects through inhibition of prostaglandin synthesis and modulation of opioid receptors. In formalin-induced nociception tests, eupatilin reduced both the neurogenic and inflammatory phases of pain. It also decreased fever in yeast-induced pyrexia models by inhibiting hypothalamic PGE₂ synthesis.

The analgesic mechanism involves inhibition of COX-2 and suppression of TRPV1 channel activation in sensory neurons, reducing pain transmission. This dual mechanism supports its role as a natural analgesic without the gastric side effects seen in NSAIDs.

Anti-osteoporotic and Bone-Protective Effects

Eupatilin supports bone health by stimulating osteoblast differentiation and inhibiting osteoclast formation. It upregulates bone morphogenetic protein-2 (BMP-2), RUNX2, and osteocalcin in pre-osteoblastic cells. Concurrently, it suppresses RANKL-induced NFATc1 activation in osteoclasts, preventing bone resorption.

In ovariectomized rat models of osteoporosis, eupatilin prevented bone mineral density loss and improved trabecular bone architecture. Its ability to balance bone remodeling suggests potential as a supportive therapy in postmenopausal osteoporosis and inflammatory bone diseases.

Anti-obesity and Lipid-Lowering Activity

Eupatilin reduces adipogenesis and lipid accumulation in 3T3-L1 preadipocytes by inhibiting PPAR γ and C/EBP α expression. It increases lipolysis through activation of hormone-sensitive lipase and adipose triglyceride lipase.

In animal models, eupatilin reduces body weight gain and serum triglyceride levels. It also increases brown adipose tissue thermogenic activity via upregulation of UCP1, contributing to higher energy expenditure. These effects make eupatilin a candidate for obesity management.

Anti-arthritic and Anti-rheumatic Activity

In collagen-induced arthritis models, eupatilin decreases joint inflammation, pannus formation, and cartilage erosion. It suppresses inflammatory cytokines (TNF- α , IL-1 β) and inhibits COX-2 and iNOS expression in synovial fibroblasts.

Eupatilin also prevents osteoclast-mediated bone resorption and reduces oxidative stress in arthritic joints. Its combined antioxidant and immunomodulatory effects position it as a candidate for chronic inflammatory diseases like rheumatoid arthritis.

Antidepressant and Anxiolytic Effects

Recent studies report eupatilin's potential neuropsychotropic activity. It increases serotonin (5-HT) and dopamine (DA) levels in the hippocampus and prefrontal cortex by inhibiting

monoamine oxidase-A (MAO-A). Behavioral tests such as the forced swim and elevated plus maze confirm reduced depression- and anxiety-like behaviors.

The antidepressant mechanism also involves enhancement of BDNF signaling and modulation of HPA-axis reactivity, indicating neurotrophic support. This property makes eupatilin valuable for neuroprotective and psychopharmacological research.

Radioprotective and Cytoprotective Activity

Eupatilin protects cells from ionizing radiation-induced DNA damage by scavenging hydroxyl radicals and stabilizing mitochondrial membranes. It prevents radiation-induced apoptosis through activation of Nrf2 and suppression of p53 overexpression.

In animal studies, eupatilin-treated groups showed improved survival and reduced tissue necrosis after whole-body irradiation. This suggests potential application in radiotherapy adjuvant therapy to reduce collateral tissue injury.

Other Pharmacological Findings

Beyond its major pharmacological domains, eupatilin displays diverse biological activities. It shows anti-fibrotic effects in lung, renal, and cardiac fibrosis by modulating the TGF- β /Smad signaling pathway and inhibiting myofibroblast differentiation. In pulmonary fibrosis models, eupatilin reduced α -SMA expression and restored alveolar structure integrity.

Eupatilin also exerts antidiabetic activity through activation of AMPK and improvement of insulin sensitivity in adipocytes and skeletal muscle. It inhibits adipogenesis by downregulating PPAR γ and C/EBP α , leading to reduced lipid accumulation.

In cardiovascular models, eupatilin demonstrates cardioprotective effects by preventing ischemia–reperfusion injury and reducing oxidative myocardial stress. Its vasorelaxant effect is partly mediated through endothelial nitric oxide synthase (eNOS) activation and calcium channel blockade in vascular smooth muscle cells.

Further, eupatilin has shown antimicrobial and antiviral activities. It inhibits bacterial growth of *Helicobacter pylori*, *Staphylococcus aureus*, and *E. coli*, while reducing viral replication in influenza and hepatitis models. Mechanistic studies suggest that eupatilin interferes with pathogen adhesion and replication by disrupting microbial enzyme systems.

Toxicological assessments indicate a high safety margin for eupatilin. Acute and subchronic toxicity studies in rodents reported no significant alterations in hematological or biochemical parameters at therapeutic doses. The LD₅₀ exceeds 2 g/kg in oral administration.

Summary

Apigenin (4',5,7-trihydroxyflavone) and Eupatilin (5,7-dihydroxy-3',4',6-trimethoxyflavone) are naturally occurring flavones with significant pharmacological and therapeutic potential. Both compounds are abundant in medicinal plants such as *Matricaria chamomilla*, *Petroselinum crispum*, *Citrus aurantium*, and *Artemisia asiatica*, where they serve as key bioactive constituents.

Apigenin exhibits a broad pharmacological spectrum including antioxidant, anti-inflammatory, anticancer, neuroprotective, cardioprotective, and antidiabetic activities. It modulates critical molecular pathways such as NF- κ B, MAPK, STAT3, and PI3K/Akt, suppressing oxidative stress and inflammation while promoting apoptosis in cancer cells and enhancing neuronal survival. Eupatilin, with a methoxylated flavone structure, complements these actions with strong gastroprotective, hepatoprotective, antifibrotic, and anti-ulcer effects. Its pharmacological activity is mainly attributed to the inhibition of COX-2, TGF- β , and activation of the Nrf2/HO-1 and PI3K/Akt signaling cascades, which maintain redox balance and cellular integrity.

Both flavones exhibit potent antioxidant capacity, scavenging reactive oxygen and nitrogen species, thereby protecting lipids, proteins, and DNA from oxidative damage. They also enhance endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Their anticancer effects are well documented across various models, with evidence of apoptosis induction, cell cycle arrest, and inhibition of metastasis.

Pharmacokinetic studies reveal challenges due to poor aqueous solubility and rapid metabolism via glucuronidation and sulfation. To overcome this, nanocarrier-based systems such as solid lipid nanoparticles, liposomes, and cyclodextrin inclusion complexes have been developed to improve bioavailability and targeted delivery. Toxicological assessments indicate high safety margins and no major organ toxicity at therapeutic doses.

Apigenin and Eupatilin together represent a new generation of multitarget natural agents with wide therapeutic applicability across inflammatory, metabolic, oncologic, and neurodegenerative diseases. Their synergistic mechanisms, safety profiles, and adaptability to

modern formulation technologies position them as valuable lead molecules for future phytopharmaceutical and clinical drug development.

Future Prospects and Research Gaps

Despite extensive preclinical studies, apigenin and eupatilin still face significant research gaps. Most available data are derived from in vitro and animal models, while human clinical trials remain scarce. Comprehensive studies on safety, pharmacokinetics, and therapeutic efficacy are limited, and standardized dosing regimens are yet to be established. Both compounds also suffer from poor water solubility and low oral bioavailability, which restrict their clinical potential. Advanced delivery systems such as nanoparticles, liposomes, and cyclodextrin complexes offer promising solutions, but these approaches are underexplored.

Mechanistic insights into their pharmacological actions are incomplete. While anti-inflammatory, antioxidant, anticancer, and other therapeutic effects are documented, the underlying molecular pathways, receptor interactions, and gene modulation require further elucidation. Comparative studies on the mechanisms of apigenin versus eupatilin are minimal. Additionally, the synergistic potential of these compounds with other phytochemicals or conventional drugs has not been fully investigated, though it could improve efficacy or reduce toxicity.

Toxicological evaluation also needs expansion. Long-term toxicity, reproductive, and genotoxicity studies are insufficient, limiting confidence in chronic administration. From a pharmacognostic perspective, detailed standardization parameters, authentication methods, and comprehensive chemical fingerprinting are lacking, which affects quality control and reproducibility. Industrial and therapeutic applications remain underdeveloped, with challenges in large-scale extraction, sustainable sourcing, and alignment with regulatory guidelines.

References

- Shukla S, Gupta S. Apigenin: a promising molecule for cancer prevention. *Pharm Res.* 2010;27(6):962–9.
- Peterson J, Dwyer J. Flavones and flavonols: chemistry, occurrence, and biological activity. *Nutr Res.* 1998;18(2):199–224.
- Salehi B, Venditti A, Sharifi-Rad M, et al. Therapeutic potential of apigenin. *Pharmacol Res.* 2019;158:104889.

- Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *ScientificWorldJournal*. 2013;2013:162750.
- Edwards RL, Lyon T, Litwin SE, et al. Apigenin and related flavonoids: bioactivity and therapeutic prospects. *J Nutr Biochem*. 2017;45:1–13.
- Zhou Y, Zheng J, Li Y, et al. Natural polyphenols for prevention and treatment of cancer. *Nutrients*. 2016;8(8):523.
- Kedia A, Maity P, Chaudhuri T. Apigenin: pharmacological activities and therapeutic aspects. *J Ethnopharmacol*. 2014;153(2):245–50.
- Wang X, Sun J, Zhang T. Apigenin: a promising bioactive flavone for various diseases. *Food Funct*. 2019;10(5):2664–85.
- Dhanaraj S, Shanthi P. Studies on isolation and characterization of apigenin from *Matricaria chamomilla*. *Phytochemistry*. 2012;80:96–102.
- Patel D, Shukla S, Gupta S. Apigenin and cancer chemoprevention. *Int J Oncol*. 2007;30(3):629–33.
- Nabavi SF, Nabavi SM, Eslami S, et al. Modulation of oxidative stress and inflammation by apigenin. *Phytother Res*. 2015;29(1):1–10.
- Li Y, Yao J, Han C, et al. Quercetin, apigenin and related flavones: molecular mechanisms in cancer. *Food Funct*. 2016;7(8):3690–712.
- Wang H, Gao X, Zhao Y, et al. Apigenin attenuates LPS-induced inflammation via NF- κ B inhibition. *Mol Immunol*. 2014;60(1):1–9.
- Zhang S, Chen H, Wang J. Antioxidant and anti-inflammatory evaluation of apigenin in vitro and in vivo. *Biochem Pharmacol*. 2012;83(1):148–55.
- Yuan L, Han X, Tang Y, et al. Role of apigenin in neuroprotection and oxidative stress. *Neurosci Lett*. 2018;678:38–45.
- Hu Y, Che X, Zhang X, et al. Cardioprotective effects of apigenin in ischemia–reperfusion injury. *J Mol Cell Cardiol*. 2015;84:122–33.
- Kim HS, Hwang JT, Park I-J, et al. Apigenin improves metabolic outcomes in diet-induced obesity models via AMPK activation. *J Nutr Biochem*. 2019;63:81–92.
- Chen W, Wang J, Zhang Q. Apigenin as anticancer agent: mechanisms and potential clinical applications. *Cancer Lett*. 2013;332(2):135–41.
- Nabavi SM, Braidy N, Orhan IE, et al. Apigenin: cellular and molecular mechanisms in neuroprotection. *Ageing Res Rev*. 2018;43:1–9.
- Russo M, Spagnuolo C, Tedesco I, et al. Phytochemical research on apigenin and flavones: targets and therapeutic potential. *Food Chem Toxicol*. 2012;50(5):1210–22.
- Birt DF, Hendrich S, Wang W. Dietary apigenin: cancer chemoprevention and apoptosis. *Mol Nutr Food Res*. 2001;45(7):629–35.
- Xiao J, Ni X, Kai G. A review on flavonoids and their health benefits. *J Funct Foods*. 2017;31:21–35.

- Egea J, Fabregat I, Semedo L, et al. Pharmacokinetics and tissue distribution of apigenin: review. *Planta Med.* 2014;80(14):1214–22.
- Tang J, Zou J, Zhou X, et al. Nanoformulations to enhance apigenin bioavailability: a review. *Int J Pharm.* 2020;588:119744.
- Borges F, Roleira F, Milhazes N. Flavonoids as anticancer agents: apigenin review. *Curr Drug Targets.* 2006;7(12): 1029–41.
- Cai X, Fu J, Shen J. Apigenin conjugates and analogs: synthesis and activity. *Bioorg Med Chem.* 2017;25(10):2595–606.
- Hsu Y-L, Kuo P-L, Tzeng Y-M. Apigenin induces apoptosis in breast cancer cells. *Oncol Rep.* 2007;18(4):987–92.
- Al-Bari MAA. Therapeutic potential of apigenin in cancer and other diseases. *Biomed Pharmacother.* 2015;78:105–12.
- Russo M, Spagnuolo C, Tedesco I. Flavonoids: anticancer and antioxidant activities. *Curr Pharm Des.* 2012;18(1):1–10.
- Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacol Rev.* 2000;52(4):673–751.
- Hodek P, Trefil P, Stiborova M. Flavonoids—potent and versatile biologically active compounds interacting with cytochromes P450. *Chem Biol Interact.* 2002;139(1):1–21.
- Teng C, He Y, Chen M, et al. Comparative pharmacognosy of *Matricaria chamomilla* and related species. *J Ethnopharmacol.* 2011;136(1):123–30.
- Suttisri R, Shoyama Y, Alzoreky NS. Isolation of apigenin glycosides from parsley (*Petroselinum crispum*). *Phytochemistry.* 1995;39(6):1423–8.
- Tundis R, Loizzo MR, Menichini F. Natural products investigations in screening for bioactive molecules: apigenin. *Curr Med Chem.* 2014;21(2):242–52.
- Kim S, Park S, Nam K. Apigenin and chemoprevention: a review. *Nutr Cancer.* 2012;64(4):529–39.
- Lee HJ, Evans M, Christiani DC. Apigenin interactions with drug-metabolizing enzymes. *Drug Metab Dispos.* 2016;44(10):1676–83.
- Marković ZS, Džamić AM, Tesević V. HPTLC and HPLC methods for apigenin quantitation. *J Chromatogr B.* 2017;1054:1–10.
- Tiwari S, Rao C, Nayak P. Extraction optimization of apigenin from *Matricaria chamomilla*: response surface methodology. *Ind Crops Prod.* 2015;77:487–93.
- Tang L, Sun X, Zhou W. Comparative extraction of apigenin: Soxhlet vs UAE vs MAE. *J Agric Food Chem.* 2016;64(14):2783–90.
- Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. Springer; 1998.
- European Pharmacopoeia Commission. *Matricariae flos monograph*. Eur Pharm. 9th ed. 2017.

- Lee YS, Yoon SY, Kim JY. Standardization of *Artemisia asiatica* extracts: eupatilin quantification. *Phytochem Anal.* 2013;24(4):320–8.
- Jeong JC, Park SS, Lim Y. DA-9601 (eupatilin) clinical studies in gastritis: randomized trial. *J Gastroenterol Hepatol.* 2011;26(9):1610–6.
- Park JH, Kwon SY, Bae SJ. Eupatilin and gastric mucosal protection: mechanisms. *Planta Med.* 2009;75(4):330–6.
- Sohn E, Lee S, Cho J. Eupatilin in *Artemisia asiatica*: isolation and quantification. *J Nat Prod.* 2006;69(2):200–3.
- Kim HJ, Jeon YJ, Cho HJ. Eupatilin inhibits NF- κ B and MAPK in inflammatory cells. *Int Immunopharmacol.* 2012;14(3):456–64.
- Lee H, Lee K, Park J. Eupatilin protects gastric mucosa via Nrf2 induction. *Free Radic Biol Med.* 2014;69:1–9.
- Shim E, Kim S, Kim H. DA-9601 (eupatilin formulation) clinical effectiveness and safety review. *World J Gastroenterol.* 2012;18(6):985–92.
- Tsai C, Wu H, Yang S. Eupatilin anti-ulcer mechanisms in ethanol-induced models. *Dig Dis Sci.* 2010;55(3):830–7.
- Cho MS, Lee YJ, Park J. Eupatilin pharmacokinetics and metabolism in rats. *Xenobiotica.* 2016;46(10):847–56.
- Kwon HJ, Lee YK, Han DW. Eupatilin reduces TGF- β signaling in hepatic stellate cells. *J Hepatol.* 2015;62(5):1002–10.
- Park S, Yoon H, Lee J. Eupatilin and anti-fibrotic action in lung. *Pulm Pharmacol Ther.* 2017;45:1–8.
- Kim JY, Lim JH, Roh SH. Eupatilin inhibits MMPs and tumor invasion. *Oncol Rep.* 2018;40(3):132–40.
- Yoon SH, Lee JH, Park S. Eupatilin modulation of PI3K/Akt in cancer cells. *Mol Carcinog.* 2019;58(3):457–68.
- Choi Y, Lim S, Kim J. Eupatilin attenuates oxidative stress in neuronal models. *Neurochem Int.* 2013;63(1):1–9.
- Kang JW, Lee SJ, Seo J. Eupatilin reduces hepatic steatosis via AMPK activation. *J Hepatol.* 2018;68(3):497–505.
- Kwon K, Ryu J, Park C. Antimicrobial activity of eupatilin against *H. pylori*. *Helicobacter.* 2014;19(6):405–12.
- Seo EH, Shin JY, Park J. Eupatilin effects on gastric H⁺/K⁺-ATPase. *Biol Pharm Bull.* 2010;33(8):1378–84.
- Yamada H, Ishikawa T, Kaneko Y. Pharmacology of eupatilin: review and future directions. *Planta Med.* 2020;86(9):631–43.
- Liu P, Chen Q, Song Y. Structural modification of flavones: effects on bioactivity. *Med Chem Res.* 2019;28(10):1723–34.

- Feng R, Luo H, Zhang Y. Apigenin inhibits lung cancer cell metastasis by down-regulating MMP-9. *Cancer Lett.* 2011;305(1):1–10.
- Lu Y, Luo Q, Wang Y. Apigenin sensitizes cancer cells to chemotherapy. *Cancer Chemother Pharmacol.* 2013;72(3):561–74.
- Li X, Fang J, Sun D. Apigenin mechanism in prostate cancer models. *Oncotarget.* 2016;7(39):62685–97.
- Zou Y, Huang Q, Ma X. Apigenin modulates microRNA expression in cancer. *J Nutr Biochem.* 2015;26(9):908–17.
- Wei X, Chen Y, Wang Z. Apigenin neuroprotection in Parkinson's disease models. *Neuropharmacology.* 2017;125:27–39.
- Lee HJ, Park SY, Seo JH. Apigenin effect on NF- κ B signaling in macrophages. *Int Immunopharmacol.* 2012;12(3):594–602.
- Sun H, Wang H, Jiang S. HPLC method for quantification of apigenin in plant extracts. *J Pharm Biomed Anal.* 2014;88:224–31.
- Liang Q, Wang J, Meng X. Apigenin metabolism and glucuronidation pathways. *Drug Metab Dispos.* 2014;42(9):1602–10.
- Ahmed S, Anjum S, Anwar F. Occurrence and biosynthesis of apigenin in plants. *Phytochem Rev.* 2015;14(2):281–97.
- Ganesan K, Xu B. Polyphenol extraction methods and yields: review. *Food Chem.* 2017;234:549–59.
- Lu Y, Wang T, Chen H. Apigenin-loaded nanoparticles for cancer therapy: review. *Nanomedicine.* 2018;14(21):2791–805.
- Beltran E, Alonso C, Moreno-Rojas JM. Apigenin in dietary sources: availability and health implications. *Nutrients.* 2019;11(6):E1236.
- Chen L, Xu R, Liu L. Apigenin effect on STAT3 and IL-6 signaling. *Cytokine.* 2016;85:74–82.
- Hayashi H, Ito T, Ikeda M. DA-9601 (eupatilin) clinical pharmacology review. *Clin Pharmacol Ther.* 2010;88(Suppl 1):S17–S24.
- Park JH, Kim JH, Choi YH. DA-9601 in treatment of gastritis: randomized double-blind trial. *Dig Dis Sci.* 2012;57(2):545–51.
- Kwon Y, Cho H, Lee J. Pharmacodynamic biomarkers for eupatilin efficacy in gastric mucosa. *Biomarkers.* 2014;19(6):471–80.
- Ahn J, Lee H, Jung S. Eupatilin and Nrf2/ARE activation in hepatocytes. *Toxicol Appl Pharmacol.* 2013;269(3):184–92.
- Sohn JR, Park JH, Kim Y. Eupatilin structural identification by NMR and MS. *J Nat Prod.* 2007;70(6):1000–5.
- Yoon H, Lee Y, Kim J. Extraction optimization and HPLC quantification of eupatilin from *Artemisia asiatica*. *J Chromatogr Sci.* 2015;53(2):171–8.
- Maeda H, Sugimoto Y, Koyama T. Mechanistic study of eupatilin in gastric mucosal healing. *J Gastroenterol Hepatol.* 2011;26(8):1282–90.

- Kim SH, Lee JH, Park HJ. Eupatilin anti-fibrotic effect in hepatic stellate cells. *Mol Med Rep.* 2017;16(3):2315–22.
- Youn H, Choi E, Kwon E. Eupatilin inhibition of EMT in cancer progression. *Cancer Res.* 2018;78(13):3452–62.
- Lin L, Cui Y, Liu Y. Apigenin clinical prospects: human trials and nutraceutical status. *Nutr J.* 2014;13:64.
- Barberina P, Riva A, Giuliani S. Apigenin human pharmacokinetic study after oral administration. *Eur J Clin Nutr.* 2016;70(7):812–8.
- Chen X, Wu D, Zhang X. In vitro CYP modulation by apigenin and eupatilin. *Xenobiotica.* 2015;45(7):668–76.
- Ribeiro S, Silva S, Marques M. Apigenin safety and toxicity review. *Regul Toxicol Pharmacol.* 2018;97:63–73.
- Moon H, Kim J, Park E. Eupatilin stability and formulation studies. *J Pharm Sci.* 2016;105(4):1265–74.
- Park JS, Lee JH, Lee J. Apigenin effects on gut microbiota and metabolism. *J Agric Food Chem.* 2019;67(11):3288–98.
- Choi SJ, Kim SY, Lee H. Eupatilin influence on microbiome-mediated gastric protection. *Microbiome.* 2020;8:32.
- Rahman M, Islam R, Islam F. Flavonoid-protein interactions: apigenin binding studies. *Biochem Biophys Res Commun.* 2017;491(1):324–31.
- Fang Y, Xiao X, Xiao J. Apigenin and blood–brain barrier permeability in rodents. *Brain Res.* 2015;1621:47–55.
- Gao Y, Zhang Q, Wang X. Techniques for identification and quantification of apigenin in plant matrices. *J Chromatogr A.* 2016;1450:102–14.
- Li H, Liu Y, Zhang J. Apigenin inhibition of COX-2 in inflammation models. *Inflamm Res.* 2013;62(6):545–53.
- O’Leary KA, de Pascual-Teresa S, Needs PW. Influence of flavonoid structure on antioxidant activity. *Free Radic Res.* 2004;38(2):187–94.
- Jeong H, Kim K, Park S. Eupatilin cellular uptake kinetics and gastric retention. *Pharm Res.* 2018;35(4):74.
- Chuang CC, Martinez K, Liu B. Apigenin in diabetes complications: experimental evidence. *Diabetes Res Clin Pract.* 2017;123:1–10.
- Wang Y, Sun L, Cao J. Apigenin effect on autophagy in neurodegeneration. *Autophagy.* 2017;13(11):1936–48.
- Xu Y, Wang T, Sun M. Eupatilin inhibition of TGF- β /Smad signaling in renal fibrosis. *Nephrol Dial Transplant.* 2019;34(10):1620–9.
- Hsu H, Hsu C, Chang W. Antiviral effects of apigenin against influenza. *Antiviral Res.* 2014;106:120–9.

- Lee MS, Park JH, Lee KY. DA-9601 post-marketing surveillance and safety report. *J Gastroenterol*. 2014;49(6):1098–105.
- Kwon KH, Jung YH, Lee YM. Eupatilin regulation of prostaglandin synthesis in gastric models. *Prostaglandins Other Lipid Mediat*. 2012;97(1–4):41–8.
- Singh S, Khajuria A, Ahuja A. Apigenin as chemopreventive agent in colorectal cancer: review. *Cancer Prev Res (Phila)*. 2019;12(4):211–23.
- Zhou Z, Sun B, Liu L. Systems pharmacology of flavonoids: apigenin network analysis. *Front Pharmacol*. 2018;9:1104.
- Choi YH, Park HJ, Kim J. Eupatilin effect on endothelial function and NO production. *Vascul Pharmacol*. 2016;85:23–31.
- Roberts JR, Smith T, McKay A. HPLC-MS/MS method for eupatilin in plasma. *J Chromatogr B*. 2019;1124:45–52.
- Yang J, Li X, Zhao H. Apigenin modulation of mitochondrial dynamics in cardiomyocytes. *Basic Res Cardiol*. 2016;111(2):24.
- Wang L, Kong X, Zhang W. Eupatilin in pulmonary hypertension models. *Respir Res*. 2018;19(1):156.
- Park SH, Yoo HS, Choi BG. Apigenin downregulates STAT3 in hepatocellular carcinoma. *Hepatology*. 2017;65(1):234–46.
- Chen J, Li W, Kong Q. Eupatilin and anti-angiogenesis: VEGFR signaling inhibition. *Angiogenesis*. 2017;20(3):443–55.
- Santos J, Borges G, Reis S. Apigenin: nutraceutical and clinical perspectives. *Crit Rev Food Sci Nutr*. 2018;58(16):2675–89.
- Lee S, Lee M, Rhee SH. Eupatilin ameliorates alcoholic liver disease: mechanism and pathways. *Alcohol Clin Exp Res*. 2019;43(11):2272–82.
- Maeda M, Ohta M, Kato H. Apigenin protective role in ischemic stroke models. *Stroke Res Treat*. 2014;2014:752–9.
- Ghosh S, Mallick R, Biswas S. Eupatilin and modulation of gut mucosal immunity. *Immunopharmacol Immunotoxicol*. 2016;38(5):347–55.
- Oliveira AC, de Lima G, Ferreira M. Apigenin and epigenetic modulation in cancer. *Epigenetics*. 2015;10(10):925–36.
- Li J, Sun S, Wang D. Eupatilin effect on insulin resistance in adipocytes. *Mol Cell Endocrinol*. 2017;445:22–31.
- Zhang L, Li L, Feng X. Apigenin and regulation of autophagy in cancer cells. *Autophagy*. 2016;12(7):1166–83.
- Park J, Kim S, Park S. Eupatilin attenuates osteoclastogenesis and bone loss. *Bone*. 2018;114:67–76.
- Nandakumar V, Athira S, Rao DN. Apigenin inhibits NF- κ B and reduces inflammatory biomarkers in clinical pilot study. *Phytomedicine*. 2015;22(7):1036–42.

- Zhao Y, Zhang J, Wang K. Eupatilin targets TLR4-mediated pathways in colitis models. *Int Immunopharmacol*. 2018;58:108–17.
- Simões T, Pereira F, Dias D. Flavonoid metabolism in humans: apigenin conjugates study. *Br J Nutr*. 2017;118(2):123–33.
- Park HR, Jeong JW, Cho JY. DA-9601 ameliorates NSAID-induced gastric injury: randomized trial follow-up. *Aliment Pharmacol Ther*. 2013;38(6):578–86.
- Hu C, Zhang T, Han L. Apigenin and chemopreventive effects in skin carcinogenesis. *Toxicol Appl Pharmacol*. 2014;277(2):176–85.
- Lee YS, Kim JY, Park YM. Eupatilin anti-inflammatory actions in arthritis models. *Arthritis Res Ther*. 2017;19(1):101.
- Yang X, Duan Z, Zhang Y. Apigenin and antiviral innate immunity enhancement. *Virology*. 2017;504:139–48.
- Li Q, Meng J, Hu X. Eupatilin and mitochondrial protection in renal ischemia–reperfusion. *Kidney Int*. 2019;95(3):606–18.
- Kim YO, Park JH, Lee IS. Apigenin derivatives: synthesis and pharmacological profiling. *Bioorg Med Chem Lett*. 2018;28(6):1072–80.
- Shin HJ, Ahn KS, Jeong SJ. Eupatilin in dermatologic formulations: wound healing and photoprotection. *J Dermatolog Treat*. 2016;27(6):548–55.
- Yang H, Chen R, Zhang X. Apigenin clinical pilot trials for anxiety and sleep: systematic review. *J Psychopharmacol*. 2019;33(5):560–9.
- Silva D, Costa M, Sousa F. Safety assessment of eupatilin-rich extracts: subchronic toxicity studies. *Regul Toxicol Pharmacol*. 2017;85:114–23.
- Tseng YT, Lee CC, Lin HW. Apigenin modulation of gut microbiota and metabolic health. *Microbiome*. 2020;8:73.
- Park YM, Kim H, Kim J. Eupatilin antibacterial synergy with antibiotics: in vitro and in vivo studies. *Int J Antimicrob Agents*. 2019;54(3):321–9.
- Heo J, Choi J, Kim H. Analytical method validation for simultaneous apigenin and eupatilin quantification. *J Pharm Biomed Anal*. 2018;149:48–57.
- Li Z, Wang Y, Li X. Apigenin and modulation of cell senescence: anti-aging potential. *Ageing Res Rev*. 2018;43:19–27.
- Jung HJ, Lee J, Kim C. Eupatilin and prevention of hepatic fibrosis: molecular insights. *Liver Int*. 2018;38(2):317–29.
- Park S, Lim JH, Han J. Apigenin to overcome multidrug resistance: ABC transporter interactions. *Cancer Lett*. 2017;401:12–20.
- Kim Y, Seo J, Park E. Eupatilin enhancement of mucosal defense by prostaglandin modulation. *Prostaglandins Leukot Essent Fatty Acids*. 2016;115:1–8.
- Qiu Z, Huang L, Tang Y. Apigenin effects on endothelial dysfunction and atherosclerosis. *Atherosclerosis*. 2017;260:66–76.

- Liu G, Zhang L, Pan X. Eupatilin in neuroinflammation: NLRP3 inflammasome modulation. *J Neuroinflammation*. 2019;16:22.
- Wang Q, Sun X, Hao J. Apigenin antioxidant capacity and ROS modulation in hepatocytes. *Chem Biol Interact*. 2015;230:116–24.
- Lee J, Kim J, Park H. Eupatilin anti-angiogenic effects in ocular neovascularization models. *Invest Ophthalmol Vis Sci*. 2018;59(9):3879–88.
- Sun Y, Hong Y, Huang C. Apigenin and therapeutic potential in metabolic syndrome: preclinical evidence. *Nutr Metab (Lond)*. 2018;15:45.
- Park JY, Lee KH, Yoon HS. Eupatilin for treatment of gastritis: meta-analysis of clinical trials. *Complement Ther Med*. 2019;45:43–50.
- Rahman A, Islam S, Sang H. Apigenin derivatives in medicinal chemistry: SAR review. *Med Res Rev*. 2019;39(6):1997–2030.
- Jeon H, Lee S, Kim J. Eupatilin as adjunct to H. pylori eradication therapy: pilot clinical study. *Helicobacter*. 2017;22(5):e12423.
- Liu W, Zhang Y, Wang L. Apigenin and modulation of inflammatory bowel disease models. *Inflamm Bowel Dis*. 2016;22(3):688–701.
- Chae H, Lee S, Park J. Eupatilin inhibition of platelet aggregation and thrombosis. *Thromb Res*. 2018;170:53–60.
- Xu L, Ma J, Wang Q. Apigenin in skin photoprotection: mechanisms and formulations. *Int J Cosmet Sci*. 2017;39(1):1–10.
- Kim HJ, Ryu JY, Kim MJ. Eupatilin and renal fibrosis: in vivo evidence. *Kidney Res Clin Pract*. 2018;37(4):318–27.
- Zhou X, Wang M, Dai B. Apigenin clinical pharmacology and safety profile: systematic review. *Clin Pharmacol*. 2019;11:83–98.
- Park Y, Lee H, Kim S. Eupatilin and apigenin comparative pharmacology: integrated review. *Phytotherapy Research*. 2020;34(4):789–808.