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SALICIN IN MODERN DRUG DISCOVERY: A COMPREHENSIVE REVIEW OF PHARMACOKINETICS, ETHNOPHARMACOLOGY, AND CLINICAL APPLICATIONS

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ABSTRACT

Background: Salicin, a prominent phenolic glycoside derived from *Salix* species, has traditionally been used to manage pain and inflammatory conditions. Despite its long-standing ethnomedicinal use, comprehensive knowledge regarding its pharmacokinetics, bioavailability, analytical characterization, and clinical potential remains limited. This review aims to consolidate current knowledge on salicin, encompassing its botanical sources, ethnopharmacological relevance, pharmacokinetics and bioavailability, analytical methodologies, and clinical evidence. **Methods:** A thorough literature search was performed using PubMed, Scopus, Web of Science, and Google Scholar. Studies reporting on salicin's chemistry, traditional uses, pharmacological mechanisms, bioavailability, analytical profiling, and clinical trials were included. Relevant data were critically appraised and synthesized to provide an integrated overview. **Results and Findings:** *Salix* species are the primary sources of salicin, and ethnomedicinal evidence supports their use for musculoskeletal and inflammatory disorders. Pharmacokinetic studies indicate that formulation and gut microbiota significantly influence bioavailability, with rapid hydrolysis to saligenin followed by metabolism to salicylic acid. Advances in analytical methods, including spectroscopic techniques, LC-MS, and HPLC, enable precise quantification and standardization. Although large-scale, long-term trials are lacking, existing clinical studies demonstrate notable anti-inflammatory and analgesic effects with a favourable safety profile. **Conclusion:** Salicin holds considerable therapeutic promise, bridging traditional knowledge and modern pharmacology. Future studies should improve bioavailability, standardize analyses, and conduct robust clinical trials to confirm dosing, efficacy, and safety.

INTRODUCTION

Salicin ($C_{13}H_{18}O_7$) is a phenolic β-glycoside composed of salicyl alcohol linked to a β-D-glucose unit through a β-1,1′-O-glycosidic bond. This structure confers water solubility and biological activity, enabling hydrolysis to saligenin and subsequent conversion to salicylic acid, the metabolite

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responsible for its analgesic and anti-inflammatory effects [1, 2]. Salicin occurs predominantly in species of *Populus* (poplars) and *Salix* (willows), where it accumulates in substantial amounts, particularly within the bark and leaves. These plant parts have historically served as the principal sources for traditional medicinal preparations. Beyond these genera, traces of salicin have also been identified in several other ethnomedicinal plants, although at lower concentrations, thereby contributing to its broader pharmacognostic significance [3].

This wide yet uneven distribution reflects its deep-rooted role in traditional systems of medicine, where willow and poplar extracts have long been prescribed for the relief of pain, fever, and inflammatory conditions, ultimately laying the foundation for its recognition as a natural precursor to modern anti-inflammatory therapeutics [4]. Early in the 19th century, salicin was isolated and later served as the structural basis for acetylsalicylic acid (aspirin), one of the most widely used medications worldwide. Its discovery marks a significant turning point in the conversion of natural products into contemporary pharmaceuticals [5, 6]. Pharmacologically speaking, salicin is hydrolysed by intestinal β-glucosidases to produce glucose and the aglycone saligenin in the gastrointestinal tract [7]. The intestinal mucosa easily absorbs the released saligenin, which is then carried to the liver. It undergoes oxidation there to produce salicylic acid, the main bioactive metabolite. The primary cause of salicin's distinctive pharmacological properties, including its anti-inflammatory, analgesic, and antipyretic effects, is salicylic acid. This pathway links traditional medicine with modern drug development by demonstrating salicin’s therapeutic role and its function as the natural precursor of aspirin [8].

The therapeutic action of salicylic acid, the active metabolite of salicin, is primarily attributed to its inhibition of cyclooxygenase (COX) enzymes, thereby reducing prostaglandin biosynthesis and modulating inflammatory pathways [9]. Beyond its well-established analgesic effects, salicin is a phenolic glycoside, a secondary metabolite known for its immunomodulatory, antioxidant, and cytoprotective properties in humans and for serving defensive functions in plants [10]. Owing to these pharmacological properties, salicin-containing preparations, such as bark decoctions, tinctures, and powders, have been widely used in traditional medicine to relieve fever, pain, and inflammatory disorders. The attached sugar moiety increases its polarity and water solubility, facilitating efficient extraction with polar solvents such as boiling water and ethanol, which historically have contributed to its therapeutic accessibility [11]. Salicin, the key bioactive in willow bark, long used for pain and fever relief, was eventually identified. Crucially, the pharmacological precursor for the production of acetylsalicylic acid (aspirin) is salicylic acid, which is derived from salicin [12]. Research on salicin is important for several reasons. First, it underscores the translational bridge between ethnomedicine and modern pharmacology, illustrating how traditional remedies have guided drug discovery [13]. Second, investigations into salicin demonstrate the potential of natural products as lead compounds for developing safer anti-inflammatory agents [14]. Notably, crude willow bark extracts containing salicin exhibit fewer gastrointestinal adverse effects than synthetic NSAIDs, owing to the synergistic action of accompanying flavonoids and polyphenols with antioxidant and cytoprotective properties [15]. Contemporary pharmacological studies reveal that salicin and its metabolites exert multiple biological actions, including suppression of pro-inflammatory cytokines, modulation of nuclear factor kappa B (NF-κB) signaling, and inhibition of cyclooxygenase (COX) enzymes [7]. Owing to these pleiotropic mechanisms, salicin and related phytochemicals have particular therapeutic value in managing chronic inflammatory disorders, in which immune dysregulation and oxidative stress play central roles [16].

Methodology selection strategies

This comprehensive overview approach was employed to select methods for evaluating salicin in contemporary drug discovery, linking traditional knowledge with preclinical and clinical research. Extensive literature searches were conducted across multiple databases, including PubMed, Scopus, Web of Science, Embase, and Google Scholar. Ethnopharmacological data were selected based on documented traditional use and botanical authenticity. Analytical and pharmacokinetic methodologies emphasized validated LC-MS techniques, supported by relevant in vitro and in vivo models to characterize absorption, metabolism, and systemic exposure. Preclinical pharmacological and toxicological studies followed established protocols to elucidate mechanisms of action and assess safety. Clinical methods prioritized controlled study designs, pharmacokinetic–pharmacodynamic integration, and regulatory compliance to ensure translational relevance and reliability of findings.

Taxonomic Placement and Evolutionary Background

The family Salicaceae (order Malpighiales), centered on the genus *Salix L.*, is an ecologically and economically important group of woody angiosperms. Once limited to *Salix and Populus*, molecular phylogenetic studies have expanded the family to over 50 genera and 1,000 species [17-19]. With 400–500 species, *Salix* remains the largest and most taxonomically complex genus, characterized by polyploidy, morphological plasticity, and frequent hybridization, which complicate species delimitation. Consequently, modern classifications increasingly rely on molecular markers (e.g., ITS, matK, chloroplast genomes) [20-22]. Willows are deciduous, dioecious shrubs or trees bearing unisexual, catkin-like flowers without petals and simple, alternate, serrated leaves; their wind-dispersed seeds are produced in small capsules. The genus is divided into sections (e.g., *Amygdalinae, Helix, Daphnella, Fragiles*) based on catkin, nectary, and bud-scale traits [23-25]. Salix is widely distributed across the Northern Hemisphere, with the highest diversity in East Asia, North America, and Eurasia; China represents the global center of diversity. The genus spans growth forms from large riparian trees to dwarf alpine and arctic species [26,27] (Table 1). Populus, the closest relative of Salix, includes approximately 30–40 species of major ecological and economic value. Other Salicaceae genera, such as Chosenia, Casearia, Flacourtia, Idesia, and Xylosma, contribute additional ornamental, ethnomedicinal, and pharmacological value. Overall, the taxonomy and distribution of Salix highlight its exceptional ecological plasticity and importance in ecosystems, phytomedicine, bioenergy, restoration, and phytoremediation [28-30].

Table 1: Natural medicinal plants rich in salicin and their botanical habitat.

Plant species	Common Name	Plant part(s) rich in salicin	Salicin content (%)	Geographical habitat	Optimal Collection time (Season)	Ref.
Salix alba	White willow	Bark	0.012	Europe, Western Asia, North Africa	Early spring	[4,31]
Salix purpurea	Purple willow	young shoots	3–9	Central & Southern Europe	Spring and early summer	[32]
Salix fragilis	Crack willow	Bark	2–8	Europe, Central Asia	Early spring	[33]
Salix daphnoides	Violet willow	Bark	4–12	Central & Eastern Europe	Spring	[34]
Salix pentandra	Bay willow	Bark	3–8	Northern & Central Europe, Scandinavia	Spring	[35]
Salix nigra	Black willow	Bark	1–5	Eastern North America (USA, Canada)	Late spring	[36]
Salix matsudana	Chinese willow	Bark, leaves	2–6	China, Korea, Japan	Spring to early summer	[37]
Salix tetrasperma	Indian willow	Bark	2–7	India, Pakistan, Bangladesh, Sri Lanka	Monsoon and post-monsoon (June–September)	[38]
Populus tremula	European aspen	Bark	0.5–3	Europe, Siberia	Early spring	[39]
Populus balsamifera	Balsam poplar	Buds, bark	1–4	Canada, USA	Spring	[36]
Populus nigra	Black poplar	Bark	1–5	Europe, West Asia, North Africa	Early spring	[40]
Salix caprea	Goat willow	Bark	1–4	Europe, Western Asia	Spring	[21]
Salix babylonica	Weeping willow	Bark	1–3	China	Spring and autumn	[41]
Salix humboldtiana	Humboldt willow	Bark	1–4	South America	Rainy season	[42]

Isolation of Salicin and Analytical Approaches

Extensive studies have focused on isolating salicin from *Salix* and related species, showing that yields depend on plant part, extraction method, and harvest season. Traditional approaches such as maceration and decoction with aqueous or hydroalcoholic solvents remain in use but are often slow and yield only moderate recovery [42]. In contrast, modern techniques such as microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) improve efficiency by shortening extraction time and enhancing yield. Comparative analyses of willow species report salicin levels ranging from about 2.0 to 20.1 mg/g extract, with *Salix purpurea* leaf extracts under UAE conditions showing the highest concentrations, significantly exceeding those obtained through conventional methods [34] (Table 2).

Purification of salicin is commonly achieved by liquid–liquid partitioning, chromatographic separation on silica or Sephadex columns, and, occasionally, preparative high-performance liquid chromatography (HPLC) to obtain pure fractions [43]. Salicin is primarily quantified using validated reversed-phase HPLC methods coupled with UV or diode-array detection. For extract quality control, analyses typically employ C18 columns with a phosphate buffer (pH 3–4) and methanol or acetonitrile as the mobile phase, with detection at 265–280 nm, ensuring high resolution and reproducible results [44].

Advanced techniques such as LC-MS/MS are particularly suited to pharmacokinetic and bioanalytical studies in complex biological matrices, as they offer highly sensitive detection at nanogram-per-milliliter levels [45, 46]. For rapid fingerprinting and semi-quantitative evaluation, high-performance thin-layer chromatography (HPTLC) and other complementary methods remain useful, particularly in botanical authentication and quality control [47]. Despite these advances, reported salicin yields remain highly variable across species and plant parts, underscoring the importance of standardized extraction protocols, seasonal harvest data, and validated analytical methods in comparative phytochemical studies [48].

Ethnopharmacological Perspective

Salicin, the primary salicinoid glycoside from *Salix* species, has been used for centuries in traditional medicine. Ancient Egyptian texts noted willow bark for pain and fever, while Hippocrates, Galen, and other Greek and Roman physicians prescribed it for rheumatism and febrile conditions [55]. Ayurvedic scriptures describe comparable formulations for managing fever and musculoskeletal discomfort, while the Chinese *materia medica* recommends willow-based preparations for hot, inflammatory disorders and joint pain. The antipyretic and analgesic effects of willow were thus empirically acknowledged, as evidenced by parallel therapeutic applications documented across diverse medical traditions. This cross-cultural significance is further reinforced by folk medicine practices worldwide, in which willow bark infusions and decoctions were widely used in Europe to relieve intermittent fevers, arthritis, and headaches [56]. Indigenous North American groups, including the Cree, Chippewa, and Mohawk, traditionally prepared willow bark as teas, poultices, and powders to reduce fever, relieve pain, and promote wound healing. In East Asia, bark infusions were commonly employed for rheumatism and respiratory ailments [57, 58].

The therapeutic outcomes largely depended on preparation methods, such as crude bark powders, and poultices were applied topically or consumed orally. Tinctures yielded hydroalcoholic extracts that remain integral to standardized phytomedicines, and decoctions facilitated the extraction of both salicinoids and polyphenols [57]. Modern pharmacopoeias now support these traditional applications; for instance, the European Medicines Agency (EMA) recommends standardized daily doses of 60–240 mg of salicin [57], depending on the formulation.

Recent phytochemical investigations have broadened this ethnopharmacological foundation by identifying novel salicin derivatives and co-occurring phenolic compounds [59]. High-resolution analyses have confirmed the presence of salicin, along with related glycosides such as salicortin, tremulacin, and catechin-linked conjugates [59].

Notably, a 2024 study on *Salix tetrasperma* reported ten previously undescribed salicin derivatives, several of which demonstrated significant inhibition of pro-inflammatory cytokine and nitric oxide production in macrophages. These findings indicate that the therapeutic activity of willow bark arises from a more diverse spectrum of metabolites than was historically recognized [60, 61]. Investigations into lesser-known taxa like *Salix schwerinii* and *Salix kochiana*, which were both found to have detectable salicin levels and strong

antioxidant activity, broadened the pharmacological repertoire of willow species [62]. Salicin is a prodrug that hydrolyses to salicyl alcohol, which then undergoes oxidation to salicylic acid, the active metabolite that inhibits cyclooxygenase and exhibits analgesic and antipyretic effects, according to pharmacokinetic data [60].

More recently, studies of diabetic neuropathy have shown that salicin decreased oxidative and inflammatory biomarkers, improved behavioural performance, and increased nerve conduction, suggesting potential neuroprotective benefits beyond its traditional analgesic function **(Table 3).**

Standardized willow bark extracts are still used in clinical trials to treat musculoskeletal conditions, particularly osteoarthritis and low back pain, although treatment outcomes vary with salicin and extract composition [63].

Table 2: Analytical profile of Salicin content from different Taxus and their key findings.

Plant Material (Part & Species)	**Extraction & Conditions**	**Reported Salicin Content**	**Analytical Method**	**Key Findings**	**Ref.**
Bark — *Salix spp*. (12 taxa)	Routine pharmacopoeia extraction; 1- and 2-year growth; autumn and spring collection	0.8–126 mg/g dry weight	HPLC	Significant variation in salicin content among species; some taxa are unsuitable for medicinal use due to low salicin levels.	[49]
Bark & leaves — *S. Populus spp.*	Spectroscopic analysis of powdered material; no solvent extraction	Bark: 10–40 mg/g dry weight; Leaves: 100–120 mg/g dry weight	IR and Raman spectroscopy	Non-destructive methods can estimate salicylate content; bark and leaves show varying salicylate concentrations.	[50]
Bark — *Salix alba & Salix purpurea*	Ethanolic extraction	*S. alba*: 4.300 µg/mL (liquid extract); *S. purpurea*: 1.167 µg/mL (liquid extract)	HPLC	Both species exhibit antibacterial activity; S. alba shows higher salicin content.	[51]
Leaves & bark — *Salix spp*. (6 species)	Ultrasound-assisted extraction; aqueous/ethanolic solvents; 20–40 min sonication	Leaf extracts: 2.0–20.1 mg/g extract	HPLC-DAD	Ultrasound-assisted extraction increases salicin yield compared with conventional methods and provides replicate data and %RSDs for repeatability.	[52]
Willow bark extracts (*Salix spp*.)	Methanol–water (50:50) extraction; centrifugation	Not focused on yield; used for QC quantification	RP-HPLC (C18); Mobile phase: methanol:0.01 M KH_2PO_4 (pH 4.01) 15:85 v/v; Detection: 265 nm	The method provides good resolution of salicin; it is widely used for quality control.	[53]
Various Salix extracts	Extraction varies; HPTLC is used for fingerprinting and semi-quantitation	Semi-quantitative salicin band intensities can be converted to mg/g using standards	HPTLC (silica gel); mobile systems and derivatizing agents are detailed in the methods	Useful for rapid screening and botanical QC where full quantitative HPLC is not available.	[54]

Clinical pharmacokinetics, bioavailability, and toxicology aspects of salicin

Salicin, a naturally occurring β-D-glucoside of salicyl alcohol, exhibits a distinct pharmacokinetic and metabolic profile when administered orally [7]. In the stomach, it is relatively stable. Still, in the small intestine, it is hydrolysed by the gut microbiota and endogenous β-glucosidases to saligenin (salicyl alcohol), which is readily absorbed through the intestinal mucosa. Salicylic acid, the main active metabolite, is produced when saligenin undergoes fast oxidation in the liver through first-pass metabolism after absorption [56]. Compared to acetylsalicylic acid (aspirin), this biotransformation proceeds more slowly, delaying the start of the therapeutic effect. However, compared with synthetic aspirin, willow bark administration maintains sustained plasma concentrations of salicylic acid, which may help explain why it causes less stomach irritation [71].

Due to extensive first-pass metabolism and partial gastrointestinal tract breakdown, salicin's oral bioavailability is considered moderate, averaging 20–30% [72]. Unlike aspirin, which reaches peak levels within 30 to 60 minutes after ingestion, salicylic acid peak plasma concentrations are usually reached within 1.5 to 3 hours after willow bark extract ingestion [7]. Depending on dose, renal clearance, and interindividual variability, the elimination half-life of salicylic acid, derived from salicin, typically ranges from 2 to 4 hours [73]. Following its formation, salicylic acid is primarily metabolized by glucuronidation in the liver during Phase II, producing salicyl acyl glucuronide and salicyl phenolic glucuronide. Additionally, it undergoes glycine conjugation, yielding salicyluric acid, the primary metabolite found in urine [74].

Hydroxylation to gentisic acid and other dihydroxybenzoic acid derivatives are examples of minor pathways. Nearly all excretion occurs through the kidneys, and because alkalinization accelerates salicylate clearance, urine pH significantly affects clearance [75]. Salicin does not acetylate cyclooxygenase enzymes as aspirin does; rather, it inhibits prostaglandin synthesis indirectly by converting it to salicylic acid, a competitive cyclooxygenase inhibitor. Compared to aspirin, it has less antiplatelet activity and a lower risk of bleeding, which can be explained by this pharmacological difference [76].

Table 3: Ethnopharmacological Perspective of Salicin and its mechanism of action

Pharmacological Effect	Herbal botanicals	Common Name	Mechanism of Action	Biological Model Used	Ref.
Anti-inflammatory	*Salix alba*	White willow	COX-2 inhibition, NF-κB suppression, ↓ TNF-α & IL-6	In vivo: Carrageenan-induced paw edema, adjuvant arthritis in rats	[64]
Antipyretic	*Salix purpurea*	Purple willow	COX inhibition	Yeast-induced pyrexia rat model	[65]
Anti-arthritic	*Salix daphnoides*	Violet willow	Inhibition of the NF-κB pathway	Clinical: Osteoarthritis & rheumatoid arthritis patients	[57]
Antioxidant	*Salix matsudana*	Chinese willow	Downregulation of iNOS, COX-2, NF-κB, ROS scavenging	LPS-induced inflammation in mice	[66]
Cytoprotective	*Salix babylonica*	Weeping willow	Antioxidant defense, cytokine regulation, NF-κB inhibition	In vitro: ROS assays; In vivo: LPS-induced inflammatory mouse model	[67]
Wound healing	*Populus nigra*	Black poplar	ROS scavenging, NF-κB modulation, fibroblast activation	Rat wound healing	[68]
Antimicrobial	*Betula pendula*	Silver birch	Salicylates inhibit prostaglandin synthesis & bacterial growth	In vitro: bacterial inhibition	[69]
Analgesic	*Gaultheria procumbens*	Wintergreen	Salicylates inhibit COX	Pain and inflammation rat models	[70]

The toxicological and safety profile of salicin has been rigorously investigated in both preclinical and clinical studies. Within therapeutic ranges (typically 120–240 mg/day), willow bark extracts are regarded as safe and well-tolerated. Clinical trials in osteoarthritis and musculoskeletal disorders consistently demonstrate an adverse event profile comparable to placebo, with the most frequently reported effects being mild gastrointestinal symptoms such as dyspepsia, nausea, or

epigastric discomfort [77]. These occur less often than with aspirin, reflecting the absence of direct acetylation of the gastric mucosa. At supratherapeutic doses, however, salicin can precipitate salicylism, a syndrome characterized by tinnitus, dizziness, nausea, vomiting, headache, and metabolic acidosis [78]. Chronic exposure to elevated doses has been associated with nephrotoxic and hepatotoxic effects [79]. Preclinical rodent studies report a NOAEL of ~1000 mg/kg for willow bark extracts, showing no mutagenic or carcinogenic effects [80, 81]. Clinically, willow bark is contraindicated in salicylate-sensitive individuals, in those with NSAID-induced asthma, peptic ulcers, renal issues, or bleeding disorders, and is avoided in children with viral infections due to the risk of Reye's syndrome. Combining it with anticoagulants, antiplatelets, or NSAIDs heightens bleeding risk and needs close monitoring [77, 82].

Pharmacokinetic studies have established that willow bark delivers salicylic acid more gradually, with prolonged systemic availability, compared with aspirin. After therapeutic administration, plasma salicylate levels typically remain within 5–15 μg/mL, a concentration markedly lower than the >200 μg/mL normally achieved with high-dose aspirin therapy for rheumatic disorders. Nevertheless, clinical investigations consistently demonstrate that willow bark is effective in relieving osteoarthritis, chronic pain, and musculoskeletal inflammation [83]. This therapeutic efficacy is believed to result not only from salicin itself but also from the additive or synergistic contributions of polyphenols, flavonoids, and other bioactive constituents inherent to the extract. Extended clinical evaluations, lasting up to six weeks, further confirm its good tolerability, with adverse event frequencies comparable to placebo. Taken together, the distinctive pharmacokinetics, metabolic features, and favorable safety profile of salicin substantiate willow bark's role as a reliable phytopharmaceutical alternative to synthetic salicylates [84].

Biological Preclinical evidence of Salicin (*In vitro and In vivo*)

Salicin, a phenolic β-glycoside from *Salix* species, shows anti-inflammatory, antioxidant, analgesic, antipyretic, and cardiovascular benefits in preclinical and in vitro studies. It inhibits NF-κB and MAPK pathways, reducing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and increasing IL-10, thereby suppressing LPS-induced inflammation in macrophages, including RAW264.7 cells [85]. Moreover, salicin mitigates vascular endothelial dysfunction in human endothelial cells by reducing reactive oxygen species (ROS) production through downregulation of NADPH oxidase (NOX4), preserving mitochondrial membrane potential, and restoring endothelial nitric oxide synthase (eNOS) activity [86]. Extracts containing salicin have demonstrated dose-dependent antioxidant activity, including radical-scavenging and ferric-reducing capacities, in assays such as DPPH, ABTS, and FRAP. These effects are frequently amplified through synergistic interactions with co-occurring flavonoids and other phenolic compounds in the extracts [56, 87]. The therapeutic efficacy of salicin has also been demonstrated in vivo across various disease models. In collagen-induced arthritis (CIA) rats, treatment with *Salix nigra* bark extract, in which salicin is the principal bioactive constituent, resulted in a marked reduction in paw edema, arthritic scores, and histopathological joint damage. These effects were associated with reduced TNF-α, IL-1β, and IL-6 levels and with normalized oxidative stress, demonstrating salicin's combined anti-inflammatory and antioxidant actions in arthritis [88] (Figure 1). Extracts of *Salix tetrasperma* have been shown to produce dose-dependent analgesic effects in rodent models of inflammatory and neuropathic pain, including acetic acid-induced writhing, formalin, hot-plate, tail-flick, and chronic constriction injury (CCI) tests. These analgesic actions appear to be mediated, at least in part, through the suppression of NF-κB activation and the downregulation of TNF-α, COX-2, and inducible nitric oxide synthase (iNOS) expression [56]. Animal studies have confirmed the antipyretic potential of salicin, particularly in models of fever induced by yeast and endotoxins. Once hydrolyzed to saligenin and subsequently metabolized into salicylic acid, salicin lowers body temperature by suppressing hypothalamic production of prostaglandin E2 (PGE2) [60]. In addition to its fever-reducing action, salicin exhibits cardioprotective activity in both experimental and cellular systems. It safeguards endothelial function during inflammatory stress by limiting cytokine-induced oxidative damage, inhibiting NF-κB signaling, and restoring nitric oxide synthesis in endothelial cells. Evidence from clinical trials with *Salicis cortex* extract further indicates that it exerts a mild inhibitory effect on platelet aggregation, which is substantially weaker than that of low-dose aspirin, suggesting cardiovascular benefits without posing a considerable antithrombotic risk [89]. Salicin acts as a prodrug, hydrolyzing to saligenin, which then hydrolyzes to salicylic acid, thereby mediating COX inhibition and the analgesic, antipyretic, and anti-inflammatory effects of salicylic acid. It also modulates oxidative stress and

inflammatory signaling via NF-κB, MAPK, and ROS pathways. While salicin is a primary active component, many effects are attributable to the synergistic action of other phenolics and flavonoids present in *Salix* extracts [90].

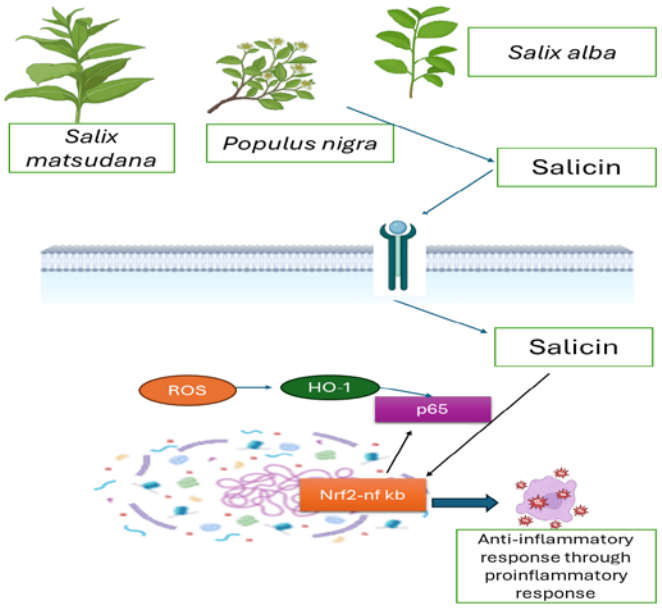

Figure 1: Salicin attenuates oxidative stress by downregulating pro-inflammatory mediators such as TNF-α, IL-6, and matrix metalloproteinases (MMPs) [63].

Clinical trials study and case studies of Salicin

Clinical trials have confirmed the effectiveness of salicin-rich willow bark extract in managing osteoarthritis (OA) and rheumatoid arthritis (RA), and in a randomized, double-blind, placebo-controlled study involving 127 patients with hip or knee OA, daily intake of a standardized extract providing 240 mg of salicin significantly reduced pain on the WOMAC pain subscale compared with placebo. The analgesic effect was comparable to that of 100 mg of diclofenac per day [57]. Similar to conventional NSAIDs in safety, the same salicin-rich extract also improved pain and physical function in a study involving 26 patients with active rheumatoid arthritis. Beyond its use in joint disorders, salicin has been investigated for chronic musculoskeletal conditions, including low back pain. In a randomized, double-blind, placebo-controlled trial, patients with acute low back pain who received willow bark extract reported significant reductions in pain and improved functional capacity, with effects comparable to those of standard analgesic treatments [91] (Table 4). In addition to its musculoskeletal benefits, salicin's effects on cardiovascular and metabolic health have been investigated. The TINSAL-CVD trial evaluated salsalate, a salicylate derivative, for its impact on coronary artery disease. While salsalate did not significantly reduce the progression of noncalcified coronary artery plaques compared with placebo, it was associated with increased albuminuria, indicating potential renal effects. Whether these observations are directly applicable to salicin remains uncertain [92]. Many studies have examined the safety, tolerability, and dose-response of salicin-containing extracts. For two weeks, both healthy volunteers and patients with chronic pain responded well to daily administration of 240 mg of standardized willow bark extract [57]. The only side effects were mild gastrointestinal symptoms and sporadic allergic reactions, particularly among individuals sensitive to salicylates [93], and the comparative studies with NSAIDs further support Salicin's therapeutic potential. In patients with rheumatoid arthritis, enteric-coated sodium salicylate and aspirin demonstrated similar safety profiles and efficacy in reducing disease activity [83].

Metabolomics profile of Salicin in drug development

Salicylic acid, the primary metabolite, then goes through Phase II conjugation reactions, such as limited hydroxylation to produce gentisic acid, glucuronidation to produce phenolic and acyl glucuronides, and glycine conjugation to form salicyluric acid [7,73]. Targeted LC-MS quantification of salicylic acid, salicyluric acid, gentisic acid, and their conjugates remains the standard analytical method, although untargeted high-resolution metabolomics is useful for identifying new metabolites [100]. Enzymatic hydrolysis with β-glucuronidase or sulfatase is commonly used to verify the presence of conjugated derivatives [101]. Pharmacokinetic studies show that systemic exposure to salicylates after therapeutic doses of willow bark is significantly lower than that seen with aspirin, with conjugates being rapidly metabolized and eliminated in the urine. Metabolomics research indicates that salicin and salicinoid consumption affects other biochemical pathways related to inflammation and metabolic control in addition to the traditional salicylate pathway. Reductions in pro-inflammatory lipid mediators like prostaglandin E_2 (PGE_2), leukotriene B_4, and downstream eicosanoid signaling products have been reported, along with changes in oxylipins derived from arachidonic acid and specific lysophospholipids. There is also evidence of alterations in phenolic conjugates and metabolites linked to oxidative stress, suggesting a more extensive metabolic footprint than salicylate exposure alone [7]. PGE_2 production in activated human immune cells is suppressed by acetylated salicinoids found in willow bark, such as 2′-O-acetylsalicortin, 3′-O-acetylsalicortin, 2′,6′-O-diacetylsalicortin, tremulacin, and lasiandrin. These

substances are broken down in vivo to produce salicin, catechol, salicylic acid, and related compounds. Therefore, salicin and its acetylated forms are regarded as important mediators of downstream biological effects, although it remains unclear to what extent they directly contribute to the human metabolomic signature [64]. Important non-salicylic metabolites of the Salicis cortex are catechol conjugates. Research in humans and rats shows that after consuming salicortin or willow bark, catechol is predominantly found in serum as glucuronide or sulfate conjugates, unless enzymatic hydrolysis is performed. When 240 mg of salicin was administered to humans, the catechol Cmax was about 1.46 mg/L (13.3 μM) with a Tmax of about 1.2 hours, indicating that catechol is an important part of the salicin-derived metabolome [102]. Recent developments in analytical techniques, such as high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) spectroscopy, have further enhanced the identification and quantitative profiling of salicin-associated metabolites and lipid mediators, allowing for a more thorough evaluation of its systemic metabolic effects [56].

Table 4: Preclinical and clinical studies of salicin and their clinical outcomes.

Study types	Populations or model	Interventions doses	Outcome	Ref.
Preclinical *(In Vitro)*	RAW264.7 macrophages	Salicin, various concentrations (10–100 μM)	↓TNF-α, ↓IL-1β, ↓IL-6, ↑IL-10; inhibition of NF-κB and MAPK pathways	[94]
Preclinical *(In Vitro)*	Human endothelial cells	Salicin, 50 μM	↓ROS, ↓NOX4, ↑eNOS activity; vascular protection	[95]
Preclinical *(In Vitro)*	DPPH, ABTS, FRAP assays	*Salix* extracts	Reduce oxidative stress	[56]
Preclinical *(In Vivo)*	Collagen-induced arthritis in rats	*Salix nigra* bark extract, 100 mg/kg, oral	↓Paw swelling, ↓arthritic score, ↓TNF-α, ↓IL-1β, ↓IL-6, and restored oxidative markers	[96]
Preclinical *(In Vivo)*	inflammatory pain in rodents (CCI, formalin, writhing, hot-plate)	*Salix tetrasperma* extract, 200–400 mg/kg, oral	↓Pain scores, ↓NF-κB, ↓TNF-α, ↓COX-2, ↓iNOS	[88]
Preclinical *(In Vivo)*	Osteoarthritis model (intra-articular injection)	Salicin-loaded PLGA microspheres	↓IRE1α phosphorylation; inhibition of ER stress; amelioration of OA progression	[56, 97]
Clinical (Human)	Osteoarthritis patients (n=78)	Standardized willow bark extract, 240 mg salicin/day	Significant reduction in WOMAC pain score; superior to placebo	[57, 65, 98]
Clinical (Human)	329 patients	Willow bark extract	Significant pain relief and improvement in physical function; no significant adverse events	[99]

Future prospective and research gaps

Salicin and its derivatives, particularly those isolated from *Salix* species, present promising opportunities for drug development. Recent studies have identified novel salicin derivatives with potent anti-inflammatory properties, suggesting their potential as lead compounds for developing new therapeutic agents. Structural modifications of these molecules may enhance efficacy while minimizing adverse effects compared to conventional nonsteroidal anti-inflammatory drugs (NSAIDs). In the context of precision medicine, salicin-based therapies could be tailored to individual patient profiles to optimize therapeutic outcomes. Achieving this requires a thorough understanding of the pharmacokinetics and pharmacodynamics of salicin metabolites. For instance, research has shown that salicin is metabolized into salicylic acid, which is subsequently conjugated to glucuronides and sulfate, processes that significantly influence its bioavailability and pharmacological activity. Such insights are critical for the development of personalized treatment strategies. Despite the promising therapeutic potential of salicin and its derivatives, there is a notable scarcity of long-term clinical trials assessing their safety and efficacy in humans. Most prior studies have been limited in

duration and scope, underscoring the need for comprehensive, extended clinical investigations to fully characterize their therapeutic profiles and monitor potential adverse effects over time. Furthermore, integrating contemporary pharmacological research with ethnomedicinal knowledge could enhance the development of salicin-based therapies. The traditional use of willow bark across diverse cultures underscores its potential therapeutic benefits. By combining this historical understanding with modern scientific approaches, researchers may uncover novel applications and optimize the clinical utility of salicin in current medical practice.

CONCLUSION

Salicin, a phenolic glycoside from *Salix* species, has long been used in traditional medicine for inflammatory conditions, and modern studies confirm its anti-inflammatory and analgesic effects, particularly in osteoarthritis and rheumatoid arthritis. After oral administration, salicin is metabolized to saligenin, which is subsequently converted to salicylic acid, the compound that mediates its therapeutic activity. Clinical evidence, including randomized controlled trials, supports the efficacy of *Salix alba* bark extracts in pain reduction. Analytical techniques such as HPLC and mass spectrometry enable standardization and quality control of salicin-containing preparations. However, long-term safety and efficacy data remain limited, highlighting the need for extended clinical trials. Integrating ethnomedicinal knowledge with contemporary pharmacological research, especially in metabolic dysregulation such as hyperlipidemia and hyperglycemia, may expand the therapeutic potential of salicin-based interventions.

ABBREVIATIONS

COX: Cyclooxygenase; NF-κB: Nuclear Factor kappa B; iNOS: Inducible Nitric Oxide Synthase; ROS: Reactive Oxygen Species; PGE_2: Prostaglandin E_2; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: Ferric Reducing Antioxidant Power; LC-MS: Liquid Chromatography–Mass Spectrometry; HPLC: High-Performance Liquid Chromatography; HPTLC: High-Performance Thin-Layer Chromatography; UAE: Ultrasound-Assisted Extraction; MAE: Microwave-Assisted Extraction; Tmax: Time to reach maximum plasma concentration; Cmax: Maximum plasma concentration; NSAIDs: Nonsteroidal Anti-Inflammatory Drugs; NOAEL: No Observed Adverse Effect Level; PLGA: Poly(lactic-co-glycolic acid); HRMS: High-Resolution Mass Spectrometry; NMR: Nuclear Magnetic Resonance; QC: Quality Control; EMA: European Medicines Agency; CVD: Cardiovascular Disease; CIA: Collagen-Induced Arthritis; CCI: Chronic Constriction Injury; mg/g: Milligrams per gram; µg/mL: Micrograms per milliliter

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Sandip Chatterjee contributed to conceptualization, study design, data collection, and preparation of the original manuscript draft. Puja Saha handled data curation, software support, data analysis, and contributed to the review of the manuscript. Dolly Rani was responsible for investigation, methodology development, drafting support, and manuscript review.

REFERENCES

[1] Huang J, Zhao D, Cui C, Hao J, Zhang Z, Guo L. Research progress and trends of phenylethanoid glycoside delivery systems. *Foods*, **11(5)**, (2022) https://doi.org/10.3390/foods11050769

[2] Liu CF. Recent advances on natural aryl-c-glycoside scaffolds: structure, bioactivities, and synthesis—a comprehensive review. *Molecules*, **27(21)**, (2022) https://doi.org/10.3390/molecules27217439

[3] Lin CR, Tsai SH, Wang C, Lee CL, Hung SW, Ting YT, et al. Willow bark (Salix spp.) used for pain relief in arthritis: a meta-analysis of randomized controlled trials. *Life*, **13(10)**, (2023) https://doi.org/10.3390/life13102058

[4] Zombori Z, Szalai G, Hamow KA, Hóvári M, Sass L, et al. Bioactive compounds in extracts from short rotation willow shoots known as pharmaceuticals and experimental demonstration of biostimulation of maize plants by these chemical complexes. *Front Plant Sci*, **16**, (2025) https://doi.org/10.3389/fpls.2025.1650824

[5] Patrono C. Fifty years with aspirin and platelets. *Br J Pharmacol*, **180(1)**, (2023) https://doi.org/10.1111/bph.15966

[6] Elwood P, Protty M, Morgan G, Pickering J, Delon C, Watkins J. Aspirin and cancer: biological mechanisms and clinical outcomes. *Open Biol*, **12(9)**, (2022) https://doi.org/10.1098/rsob.220124

[7] Geszke-Moritz M, Nowak G, Moritz M, Feist B, Nycz JE. Role of plant materials with anti-inflammatory effects in phytotherapy of osteoarthritis. *Curr Top Med Chem*, **25(1)**, (2025) https://doi.org/10.2174/0115680266297662240527105450

[8] Yeoh SC, Goh CF. Topical delivery of salicylates. *Drug Deliv Transl Res*, **12(5)**, (2022) https://doi.org/10.1007/s13346-021-00988-5

[9] Choi D, Al Baki MA, Ahmed S, Kim Y. Aspirin inhibition of prostaglandin synthesis impairs mosquito egg development. *Cells*, **11(24)**, (2022) https://doi.org/10.3390/cells11244092

[10] Johnson JB, Mani JS, Broszczak D, Prasad SS, Ekanayake CP, Strappe P. Hitting the sweet spot: a systematic review of the bioactivity and health benefits of phenolic glycosides from medicinally used plants. *Phytother Res*, **35(7)**, (2021) https://doi.org/10.1002/ptr.7042

[11] Liu CX. Overview on development of ASEAN traditional and herbal medicines. *Chin Herb Med*, **13(4)**, (2021) https://doi.org/10.1016/j.chmed.2021.09.002

[12] Santos-Gallego CG, Badimon J. Overview of aspirin and platelet biology. *Am J Cardiol*, **144**, (2021) S2-S9 https://doi.org/10.1016/j.amjcard.2020.12.018

[13] Pirintsos S, Panagiotopoulos A, Bariotakis M, Daskalakis V, Lionis C, Sourvinos G. From traditional ethnopharmacology to modern natural drug discovery: a methodology discussion and specific examples. *Molecules*, **27(13)**, (2022) https://doi.org/10.3390/molecules27134060

[14] Shrivastava R, Modi G, Satpathy PS, Bandyopadhyay S, Kumar Y, Yadav I. Natural products revolutionizing and innovative drug discovery and development strategies: healthcare challenges and future perspectives. *J Appl Bioanal*, **10(1)**, (2024) 20-38 https://doi.org/10.53555/jab.v10.002

[15] Wautier JL, Wautier MP. Pro- and anti-inflammatory prostaglandins and cytokines in humans: a mini review. *Int J Mol Sci*, **24(11)**, (2023) 9647 https://doi.org/10.3390/ijms24119647

[16] Jamtsho T, Yeshi K, Perry MJ, Loukas A, Wangchuk P. Approaches, strategies and procedures for identifying anti-inflammatory drug lead molecules from natural products. *Pharmaceuticals*, **17(3)**, (2024) 283 https://doi.org/10.3390/ph17030283

[17] Marinček P, Léveillé-Bourret É, Heiduk F, Leong J, Bailleul SM, Volf M, et al. Challenge accepted: evolutionary lineages versus taxonomic classification of North American shrub willows (Salix). *Am J Bot*, **111(7)**, (2024) e16361 https://doi.org/10.1002/ajb2.16361

[18] Ahmed SS, Rahman MO, Ali MA, Al Hemaid F, Lee J. Molecular phylogenetics and molecular dating of Arecaceae in Bangladesh inferred from matK and rbcL genes. *Bangladesh J Plant Taxon*, **30(2)**, (2023) 213-232 https://doi.org/10.3329/bjpt.v30i2.70498

[19] Ogutcen E, de Lima Ferreira P, Wagner ND, Marinček P, Leong JV, Aubona G, et al. Phylogenetic insights into the Salicaceae: the evolution of willows and beyond. *Mol Phylogenet Evol*, **199**, (2024) 108161 https://doi.org/10.1016/j.ympev.2024.108161

[20] Marchenko AM, Kuzovkina YA. Notes on the nomenclature and taxonomy of Salix fragilis (Salicaceae). *Taxon*, **71(4)**, (2022) 721-732 https://doi.org/10.1002/tax.12685

[21] Wagner ND, He L, Hörandl E. The evolutionary history, diversity, and ecology of willows (Salix L.) in the European Alps. *Diversity*, **13(4)**, (2021) 146 https://doi.org/10.3390/d13040146

[22] Zhang XJ, Liu KJ, Wang YC, He J, Wu YM, Zhang ZX. Complete chloroplast genomes of three Salix species: genome structures and phylogenetic analysis. *Forests*, **12(12)**, (2021) 1681 https://doi.org/10.3390/f12121681

[23] Achim F, Dinca L, Chira D, Raducu R, Chirca A, Murariu G. Sustainable management of willow forest landscapes: a review of ecosystem functions and conservation strategies. *Land*, **14(8)**, (2025) 1593 https://doi.org/10.3390/land14081593

[24] He L, Wagner ND, Hörandl E. Restriction-site associated DNA sequencing data reveal a radiation of willow species (Salix L., Salicaceae) in the Hengduan Mountains and adjacent areas. *J Syst Evol*, **59(1)**, (2021) 44-57 https://doi.org/10.1111/jse.12593

[25] Marchenko AM, Kuzovkina YA. Notes on the taxonomy of Salix vitellina (Salicaceae). *Plants*, **12(14)**, (2023) 2610 https://doi.org/10.3390/plants12142610

[26] Körner C. Concepts in alpine plant ecology. *Plants*, **12(14)**, (2023) 2666 https://doi.org/10.3390/plants12142666

[27] Singh BK, Delgado-Baquerizo M, Egidi E, Guirado E, Leach JE, Liu H, et al. Climate change impacts on plant pathogens, food security and paths forward. *Nat Rev Microbiol*, **21(10)**, (2023) 640-656 https://doi.org/10.1038/s41579-023-00900-7

[28] Apuli RP, Richards T, Rendón-Anaya M, Karacic A, Rönnberg-Wästljung AC, Ingvarsson PK. The genetic basis of adaptation in phenology in an introduced population of Black Cottonwood (Populus trichocarpa, Torr. & Gray). *BMC Plant Biol*, **21(1)**, (2021) 317 https://doi.org/10.1186/s12870-021-03103-5

[29] de Mestier A, Brokamp G, Celis M, Falcón-Hidalgo B, Gutiérrez J, Borsch T. Character evolution and biogeography of Casearia (Salicaceae): evidence for the South American origin of a pantropical genus and for multiple migrations to the Caribbean islands. *Taxon*, **71(2)**, (2022) 321-347 https://doi.org/10.1002/tax.12656

[30] Walczak N, Walczak Z. Analysis of the changes in the mechanical properties of branches of Salix energy plants after shearing. *Forests*, **16(2)**, (2025) 206 https://doi.org/10.3390/f16020206

[31] Saracila M, Panaite TD, Predescu NC, Untea AE, Vlaicu PA. Effect of dietary salicin standardized extract from Salix alba bark on oxidative stress biomarkers and intestinal microflora of broiler

chickens exposed to heat stress. *Agriculture*, **13(3)**, (2023) 698 https://doi.org/10.3390/agriculture13030698

[32] Zheng L, Jacquier JC, Harbourne N. Preparation of polyphenol-rich herbal beverages from white willow (Salix alba) bark with potential Alzheimer's disease inhibitory activity in silico. *Beverages*, **10(3)**, (2024) 75 https://doi.org/10.3390/beverages10030075

[33] Tienaho J, Reshamwala D, Sarjala T, Kilpeläinen P, Liimatainen J, Dou J, et al. Salix spp. bark hot water extracts show antiviral, antibacterial, and antioxidant activities—the bioactive properties of 16 clones. *Front Bioeng Biotechnol*, **9**, (2021) 797939 https://doi.org/10.3389/fbioe.2021.797939

[34] Aleman RS, Marcia J, Duque-Soto C, Lozano-Sánchez J, Montero-Fernández I, Ruano JA, et al. Effect of microwave and ultrasound-assisted extraction on the phytochemical and in vitro biological properties of willow (Salix alba) bark aqueous and ethanolic extracts. *Plants*, **12(13)**, (2023) 2533 https://doi.org/10.3390/plants12132533

[35] Antoniadou K, Herz C, Le NP, Mittermeier-Kleßinger VK, Förster N, Zander M, et al. Identification of salicylates in willow bark (Salix cortex) for targeting peripheral inflammation. *Int J Mol Sci*, **22(20)**, (2021) 11138 https://doi.org/10.3390/ijms222011138

[36] Chavan SB, Dhillon RS, Sirohi C, Keerthika A, Kumari S, Bharadwaj KK, et al. Enhancing farm income through boundary plantation of poplar (Populus deltoides): an economic analysis. *Sustainability*, **14(14)**, (2022) 8663 https://doi.org/10.3390/su14148663

[37] Ma X, Pang Z, Wu J, Zhang G, Dai Y, Zou J, et al. Seasonal pattern of stem radial growth of Salix matsudana and its response to climatic and soil factors in a semi-arid area of North China. *Global Ecol Conserv*, **28**, (2021) e01701 https://doi.org/10.1016/j.gecco.2021.e01701

[38] Reshi ZA, Husain FM, Khanam MN, Javed SB. Effect of meta-Topolin on morphological, physiochemical, and molecular dynamics during in vitro regeneration of Salix tetrasperma Roxb. *BMC Plant Biol*, **25(1)**, (2025) 121 https://doi.org/10.1186/s12870-025-06095-8

[39] Krutovsky KV, Popova AA, Yakovlev IA, Yanbaev YA, Matveev SM. Response of Pedunculate Oak (Quercus robur L.) to Adverse Environmental Conditions in Genetic and Dendrochronological Studies. *Plants*, **14(1)**, (2025) 109 https://doi.org/10.3390/plants14010109

[40] Bytner O, Drożdżek M, Laskowska A, Zawadzki J. Temperature, time, and interactions between them in relation to colour parameters of black poplar (Populus nigra L.) thermally modified in nitrogen atmosphere. *Materials*, **15(3)**, (2022) 824 https://doi.org/10.3390/ma15030824

[41] Zhang Y, Luo P, Xu J, Hou J, Zhai L. Intra-annual growth and its response to climatic factors of two Salix species under warm temperate environment. *Forests*, **13(9)**, (2022) 1441 https://doi.org/10.3390/f13091441

[42] Salgado M, Gallo LA, Torres CD, Stecconi M, Puntieri JG. Variations in growth and architecture in the range limit of Salix humboldtiana, the willow native to South America. *Botany*, **99(11)**, (2021) 713-723 https://doi.org/10.1139/cjb-2021-0017

[43] Dou J, Heinonen J, Vuorinen T, Xu C, Sainio T. Chromatographic recovery and purification of natural phytochemicals from underappreciated willow bark water extracts. *Sep Purif Technol*, **261**, (2021) 118247 https://doi.org/10.1016/j.seppur.2020.118247

[44] Baker PW, Pisano I, Nozari-Asbemarz M, Johnston C, Lindegaard K, Prendergast J, et al. Comparison of salicin and fibre contents in the barks and woody cores of willow (Salix spp.) clones as potential feedstocks for an integrated biorefinery. *Ind Crops Prod*, **225**, (2025) 120519 https://doi.org/10.1016/j.indcrop.2025.120519

[45] Chew YL, Khor MA, Lim YY. Choices of chromatographic methods as stability indicating assays for pharmaceutical products: a review. *Heliyon*, **7(3)**, (2021) https://doi.org/10.1016/j.heliyon.2021.e06553

[46] Patel HB, Dave RH, Vadariya S, Hirpara H. Development and validation for the quantification of genotoxic impurity in Mirabegron drug substance by LCMS. *Biomed Chromatogr*, **39(8)**, (2025) e70150 https://doi.org/10.1002/bmc.70150

[47] Kulkarni RN, Pandhare RB, Deshmukh VK, Mohite PB, Pawar AR. High-performance thin layer chromatography: a powerful analytical technique in pharmaceutical drug discovery. *J Pharm Biol Sci*, **9(1)**, (2021) 7 https://doi.org/10.18231/j.jpbs.2021.002

[48] Tawfeek N, Mahmoud MF, Hamdan DI, Sobeh M, Farrag N, Wink M, et al. Phytochemistry, pharmacology and medicinal uses of plants of the genus Salix: an updated review. *Front Pharmacol*, **12**, (2021) 593856 https://doi.org/10.3389/fphar.2021.593856

[49] Dou J, Ilina P, Hemming J, Malinen K, MäkkyLä H, Oliveira de Farias N, et al. Effect of hybrid type and harvesting season on phytochemistry and antibacterial activity of extracted metabolites from Salix bark. *J Agric Food Chem*, **70(9)**, (2022) 2948-2956 https://doi.org/10.1021/acs.jafc.1c08161

[50] Mazurek S, Włodarczyk M, Pielorz S, Okińczyc P, Kuś PM, Długosz G, et al. Quantification of salicylates and flavonoids in poplar bark and leaves based on IR, NIR, and Raman spectra. *Molecules*, **27(12)**, (2022) 3954 https://doi.org/10.3390/molecules27123954

[51] Farkas A. Salicin content from Salix alba L. and Salix purpurea L. extracts and its antibacterial effects. *Contrib Bot*, (2022) https://doi.org/10.24193/Contrib.Bot.57.10

[52] Gligorić E, Igić R, Teofilović B, Grujić-Letić N. Phytochemical screening of ultrasonic extracts of Salix species and molecular docking study of Salix-derived bioactive compounds targeting

pro-inflammatory cytokines. *Int J Mol Sci*, **24(14)**, (2023) 11848 https://doi.org/10.3390/ijms241411848

[53] Wiczkowski W, Marasek-Ciołakowska A, Szawara-Nowak D, Kaszubski W, Góraj-Koniarska J, Mitrus J, et al. Effect of fluridone on roots and leaf buds development in stem cuttings of Salix babylonica (L.) 'Tortuosa' and related metabolic and physiological traits. *Molecules*, **29**, (2024) 5410 https://doi.org/10.3390/molecules29225410

[54] Curtasu MV, Nørskov NP. Quantitative distribution of flavan-3-ols, procyanidins, flavonols, flavanone and salicylic acid in five varieties of organic winter dormant Salix spp. by LC-MS/MS. *Heliyon*, **10(3)**, (2024) https://doi.org/10.1016/j.heliyon.2024.e25129

[55] Rezabakhsh A, Mahmoodpoor A, Soleimanpour H. Historical perspective of aspirin: a journey from discovery to clinical practice ancient and modern history. *J Cardiovasc Thorac Res*, **13(2)**, (2021) 179 https://doi.org/10.34172/jcvtr.2021.28

[56] Gligorić E, Igić R, Čonić BS, Kladar N, Teofilović B, Grujić N. Chemical profiling and biological activities of "green" extracts of willow species (Salix L., Salicaceae): experimental and chemometric approaches. *Sustain Chem Pharm*, **32**, (2023) 100981 https://doi.org/10.1016/j.scp.2023.100981

[57] Shah N, Sapra R, Kumari M, Sadhu P, Talele C. Herbal remedies for pain: a focus on plant-based solutions. *Pharmacognosy Res*, **17(4)**, (2025) https://doi.org/10.5530/pres.20252325

[58] Li W. Cleaning and healing: an examination of the ritual of willow twigs and clean water. *Religions*, **16(4)**, (2025) 432 https://doi.org/10.3390/rel16040432

[59] Noleto-Dias C, Lomax C, Bellisai A, Ruvo G, Harflett C, Macalpine WJ, et al. Breeding novel chemistry in willow: new hetero Diels–Alder cyclodimers from arbusculoidin and salicortin suggest parallel biosynthetic pathways. *Plants*, **13(12)**, (2024) 1609 https://doi.org/10.3390/plants13121609

[60] Utari F, Itam A, Efdi M. Chemical constituents and antioxidant activity of Salix tetrasperma Roxb. *Rasayan J Chem*, **13(2)**, (2020) https://doi.org/10.31788/RJC.2020.1325547

[61] Salih E, Mgbeahuruike EE, Prévost-Monteiro S, Sipari N, Väre H, Novak B, et al. Polyphenols and phenolic glucosides in antibacterial twig extracts of naturally occurring Salix myrsinifolia (Salisb.), S. phylicifolia (L.) and S. starkeana (Willd.) and the cultivated hybrid S. x pendulina (Wender.). *Pharmaceutics*, **16(7)**, (2024) 916 https://doi.org/10.3390/pharmaceutics16070916

[62] Lee J, Song Y, Son H, Kim S, Lee KH, Bazarragchaa B, et al. Phytochemical and antioxidant characterization of extracts from unexplored medicinal plants Salix schwerinii and Salix kochiana. *Horticulturae*, **9(9)**, (2023) 955 https://doi.org/10.3390/horticulturae9090955

[63] Basu P, Basu A. In vitro and in vivo effects of flavonoids on peripheral neuropathic pain. *Molecules*, **25(5)**, (2020) 1171 https://doi.org/10.3390/molecules25051171

[64] Antoniadou K, Herz C, Le NPK, Mittermeier-Kleßinger VK, Förster N, Zander M, et al. Identification of salicylates in willow bark (Salix cortex) for targeting peripheral inflammation. *Int J Mol Sci*, **22(20)**, (2021) 11138 https://doi.org/10.3390/ijms222011138

[65] Zayed A, Salem MA, Negm WA, Ezzat SM. Validation of anti-pyretic-derived natural products and their potentials for drug discovery. *Rev Bras Farmacogn*, **33(4)**, (2023) 696-712 https://doi.org/10.1007/s43450-023-00403-9

[66] Hamed AR, Nabih HK, El-Rashedy AA, Mohamed TA, Mostafa OE, K Ali S, et al. Salvimulticanol from Salvia multicaulis suppresses LPS-induced inflammation in RAW264.7 macrophages: in vitro and in silico studies. *Biotech*, **14(5)**, (2024) 144 https://doi.org/10.1007/s13205-024-03987-8

[67] Xiao Y, Xian Y, Hu X, Qi Z. D(-)-salicin inhibits RANKL-induced osteoclast differentiation and function in vitro. *Fitoterapia*, **157**, (2022) 104981 https://doi.org/10.1016/j.fitote.2021.104981

[68] Atia A, Atmani-Kilani D, Atmani D, Ayouni K, Belkhir S, Benloukil M, et al. Wound healing potential of a formula based on Populus nigra L. flower buds extract with anti-inflammatory activity. *J Ethnopharmacol*, **331**, (2024) 118319 https://doi.org/10.1016/j.jep.2024.118319

[69] Kapadia P, Newell AS, Cunningham J, Roberts MR, Hardy JG. Extraction of high-value chemicals from plants for technical and medical applications. *Int J Mol Sci*, **23(18)**, (2022) 10334 https://doi.org/10.3390/ijms231810334

[70] Michel P, Olszewska MA. Phytochemistry and biological profile of Gaultheria procumbens L. and wintergreen essential oil: from traditional application to molecular mechanisms and therapeutic targets. *Int J Mol Sci*, **25(1)**, (2024) 565 https://doi.org/10.3390/ijms25010565

[71] Pillai RR, Sreelekshmi PB, Meera AP, Thomas S. Eco-friendly fabrication of nanocurcumin/nano iron oxide composite: enhanced in vitro anticancer activity against DLD-1 cell lines. *J Inorg Organomet Polym Mater*, **33(12)**, (2023) 3805-3814 https://doi.org/10.1007/s10904-023-02735-4

[72] Shi J, Shen L, Xiao Y, Wan C, Wang B, Zhou P, et al. Sub-inhibitory concentrations of tigecycline could attenuate the virulence of Staphylococcus aureus by inhibiting the product of α-toxin. *Microbiol Spectr*, **13(5)**, (2025) e01344-24 https://doi.org/10.1128/spectrum.01344-24

[73] Pal RS, Pal Y, Lalitha Chaitanya MVN, Mazumder A, Khurana N, Tharu PK. Discerning the multi-dimensional role of salicin: bioactive glycoside beyond analgesic: different perspectives. *Curr Drug Ther*, **19(7)**, (2024) 757-764 https://doi.org/10.2174/0115748855272391231114042540

[74] Sidlak AM, Spadaro A, Koyfman A, Long B, Sidlak A. Acute salicylate toxicity: a narrative review for emergency clinicians. *Cureus*, **17(9)**, (2025) https://doi.org/10.7759/cureus.91505

[75] Michel P. Gaultherin, a natural alternative to aspirin: a comprehensive review of molecular mechanisms, pharmacokinetics, biocompatibility, isolation techniques, and plant sources. *Int J Mol Sci*, **26(15)**, (2025) 7280 https://doi.org/10.3390/ijms26157280

[76] Tosi N, Bresciani L, Favari C, Rosi A, Dodi R, Bertelé A, et al. How does the flavan-3-ol structure condition the quali-quantitative production of phenolic metabolites? Insights from a 3-arm intervention. *Food Chem*, **491**, (2025) https://doi.org/10.1016/j.foodchem.2025.145207

[77] Andrei M, Ene N, Pintilie L, Radu N, Tomescu AJ, Raiciu AD. Multiple natural approaches of Salix alba. *Arch Microbiol Immunol*, **8(3)**, (2024) 410-427 https://doi.org/10.26502/ami.936500188

[78] Saracila M, Panaite TD, Papuc CP, Criste RD. Heat stress in broiler chickens and the effect of dietary polyphenols, with special reference to Willow (Salix spp.) bark supplements—a review. *Antioxidants*, **10(5)**, (2021) 686 https://doi.org/10.3390/antiox10050686

[79] Thongprayoon C, Petnak T, Kaewput W, Mao MA, Kovvuru K, Kanduri SR, et al. Hospitalizations for acute salicylate intoxication in the United States. *J Clin Med*, **9(8)**, (2020) 2638 https://doi.org/10.3390/jcm9082638

[80] Visagie JL, Aruwajoye GS, van der Sluis R. Pharmacokinetics of aspirin: evaluating shortcomings in the literature. *Expert Opin Drug Metab Toxicol*, **20(8)**, (2024) 727-740 https://doi.org/10.1080/17425255.2024.2386368

[81] González-Alamilla EN, Gonzalez-Cortazar M, Valladares-Carranza B, Rivas-Jacobo MA, Herrera-Corredor CA, Ojeda-Ramírez D, et al. Chemical constituents of Salix babylonica L. and their antibacterial activity against gram-positive and gram-negative animal bacteria. *Molecules*, **24(16)**, (2019) 2992 https://doi.org/10.3390/molecules24162992

[82] De Souza DP, Eisenbies MH, Volk TA. Growing season harvests of shrub willow (Salix spp.) have higher nutrient removals and lower yields compared to dormant-season harvests. *Forests*, **13(11)**, (2022) 1936 https://doi.org/10.3390/f13111936

[83] Fares S, Omar M, Laurence A, Abu-Baker S, Shaza A, Fadi H, et al. Over-the-counter anti-inflammatory supplements for adjunctive rheumatoid arthritis therapy: a comprehensive narrative review. *Aging Dis*, **16(1)**, (2024) 408 http://dx.doi.org/10.14336/AD.2024.0131

[84] Houben K, Delahaut P, Scippo ML, Daeseleire E, Croubels S, Urbain B, et al. Salicylic acid residues in products of animal origin. (2024) https://doi.org/10.2903/fr.efsa.2024.FR-0015

[85] Zheng Y, Chen J, Wu X, Zhang X, Hu C, Kang Y, et al. Enhanced anti-inflammatory effects of silibinin and capsaicin combination in lipopolysaccharide-induced RAW264.7 cells by inhibiting NF-κB and MAPK activation. *Front Chem*, **10**, (2022) 934541 https://doi.org/10.3389/fchem.2022.934541

[86] Park JH, Lee TK, Kim DW, Sim H, Lee JC, Kim JD, et al. Neuroprotective effects of salicin in a gerbil model of transient forebrain ischemia by attenuating oxidative stress and activating PI3K/Akt/GSK3β pathway. *Antioxidants*, **10(4)**, (2021) 629 https://doi.org/10.3390/antiox10040629

[87] Piątczak E, Dybowska M, Płuciennik E, Kośla K, Kolniak-Ostek J, Kalinowska-Lis U. Identification and accumulation of phenolic compounds in the leaves and bark of Salix alba (L.) and their biological potential. *Biomolecules*, **10(10)**, (2020) 1391 https://doi.org/10.3390/biom10101391

[88] Zhang Z, Wang W, Xu P, Cui Q, Yang X, Hassan AE. Synthesis and anti-inflammatory activities of two new N-acetyl glucosamine derivatives. *Sci Rep*, **14(1)**, (2024) 11079 https://doi.org/10.1038/s41598-024-61780-2

[89] Kim KA, Kang DM, Ko YJ, Ra MJ, Jung SM, Yu JN, et al. Chaenomelin, a new phenolic glycoside, and anti-Helicobacter pylori phenolic compounds from the leaves of Salix chaenomeloides. *Plants*, **13(5)**, (2024) 701 https://doi.org/10.3390/plants13050701

[90] Farag SA, El-Keredy A, Abd El Gawad SA, Swelum AA, Tellez-Isaias G, Abouzeid AE. Impacts of willow (Salix babylonica L.) leaf extract on growth, cecal microbial population, and blood biochemical parameters of broilers. *Poult Sci*, **103(3)**, (2024) 103386 https://doi.org/10.1016/j.psj.2023.103386

[91] Kayser L, Gruber TO, Aas G, Jürgenliemk G. Pharmaceutical potential of willow leaves in terms of salicylic alcohol content. *Biochem Syst Ecol*, **124**, (2025) 105169 https://doi.org/10.1016/j.bse.2025.105169

[92] Lu W, Liao X, Jiang Y, Luo B, Chen L, Lin Y. Association between inflammatory burden index and prognosis in patients with coronary heart disease: a retrospective study. *PLoS One*, **20(7)**, (2025) e0325287 https://doi.org/10.1371/journal.pone.0325287

[93] Mahmoud AH, Sloutski A, Fu S, Luo H, Katevatis Z, Wong R, et al. An innovative injectable and retrievable drug delivery system for endodontic therapy. *J Endod*, (2025) https://doi.org/10.1016/j.joen.2025.08.001

[94] Zhuang HM, Yao H. Pharmacological effects, molecular mechanisms, and pharmacokinetics of benzoylaconine: a systematic review. *Front Pharmacol*, **16**, (2025) 1571153 https://doi.org/10.3389/fphar.2025.1571153

[95] Tomović Pavlović K, Anderluh M, Šmelcerović A. Mechanisms of beneficial effects of DPP-4 inhibitors as a promising perspective for the prevention/treatment of the disruption of cardio-cerebrovascular homeostasis. *Front Pharmacol*, **16**, (2025) 1642333 https://doi.org/10.3389/fphar.2025.1642333

[96] Wang HY, Liu XJ, Yin MZ, Cui SJ, Liang HY, Xu ZH. Transcriptomic analysis of anthocyanin degradation in Salix alba bark: insights into seasonal adaptation and forestry applications. *Forests*, **16(10)**, (2025) 1598 https://doi.org/10.3390/f16101598

[97] Mahmoud MF, Rezq S, Alsemeh AE, Abdelfattah MA, El-Shazly AM, Daoud R, et al. RETRACTED: Potamogeton perfoliatus L. Extract Attenuates Neuroinflammation and Neuropathic Pain in Sciatic Nerve Chronic Constriction Injury-Induced Peripheral Neuropathy in Rats. *Front Pharmacol*, **12**, (2021) 799444 https://doi.org/10.3389/fphar.2021.799444

[98] Nhung TT, Quoc LP. Analgesic and antipyretic activities of ethanol extract of Gardenia jasminoides Ellis fruits in mice. *Trop J Nat Prod Res*, **7(10)**, (2023) 4902-4907 https://doi.org/10.26538/tjnpr/v7i10.27

[99] Ghosh N, Pathak S, Malsawmdawngkimi, Kumar G, Gull A. Natural products and traditional herbal medicines as managerial therapies to combat rheumatoid arthritis. *Clin Transl Metab*, **22(1)**, (2024) 2 https://doi.org/10.1007/s12018-024-09290-7

[100] Shinde Y, Deokar G. Regulation of gut microbiota by herbal medicines. *Curr Drug Metab*, **25(2)**, (2024) 110-127 https://doi.org/10.2174/0113892002287336240328083220

[101] Ebmeyer J, Najjar A, Lange D, Boettcher M, Voß S, Brandmair K, et al. Next generation risk assessment: an ab initio case study to assess the systemic safety of the cosmetic ingredient, benzyl salicylate, after dermal exposure. *Front Pharmacol*, **15**, (2024) 1345992 https://doi.org/10.3389/fphar.2024.1345992

[102] Gruber TO, Kuck K, Heilmann J, Jürgenliemk G. Short Lecture 3 "HCH-catechin isolated from Salix cinerea L. significantly reduces the TNFα-induced ICAM-1 expression in vitro". *Planta Med*, **88(15)**, (2022) https://doi.org/10.1055/s-0042-1758884