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REVIEW ARTICLE

A Review on Phytochemistry, and Pharmacological Activities of *Lithospermum officinale* L.

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Abstract: *Lithospermum officinale* L. (Boraginaceae) is used across Eurasian and regional ethnomedicinal systems for the management of urolithiasis, nephritic complications, gastrointestinal spasms, acute cutaneous trauma, and eruptive fevers. The therapeutic efficacy of this perennial herb is due to specialized secondary metabolites concentrated within subterranean roots and calcified nutlets. The main bioactive fractions consist of lipophilic monomeric and dimeric naphthoquinones (shikonin, acetylshikonin, and alkannin isomers), polyphenolic cinnamic acid derivatives (lithospermic acid, rosmarinic acid, and chlorogenic acid), flavonoids, and seed-derived polyunsaturated fatty acids characterized by unusual concentrations of stearidonic and γ-linolenic acids. Experimental *in vitro* and *in vivo* pharmacological evaluations indicate targeted antioxidant, anti-inflammatory, antimicrobial, burn-wound restorative, and antithyrotropic properties. The capacity of crude extracts and isolated rosmarinic acid to attenuate microglial neuroinflammation, paired with the vascular and re-epithelialization properties of naphthoquinones in cutaneous models, corroborates ancestral empirical uses. The identification of hepatotoxic 1,2-dehydropyrrolizidine alkaloids presents clear safety limitations that necessitate qualitative standardization. Resolving biomass yield fluctuations and metabolite-divergence profiles across the Lithospermeae tribe demands the integration of pharmaphylogenetic taxonomy with fungal endophyte bioprospecting. Exploiting mutualistic endophytes provides a viable biotechnological platform for producing enantiomerically pure naphthoquinones independently of natural populations.

Keywords: *Lithospermum officinale*; Shikonin derivatives; Rosmarinic acid; Pyrrolizidine alkaloids; Pharmaphylogeny.

1. Introduction

Phytomedicine plays a vital role in global healthcare systems, serving as fertile grounds for discovering lead chemical moieties [1]. Plants synthesize an array of secondary metabolites derived from primary metabolic pathways, yielding structural diversity that cannot be easily replicated by total synthetic routes [2]. Among historically documented angiosperms, *Lithospermum officinale* L., an herbaceous perennial classified within the Boraginaceae family, occupies a prominent position [3, 4]. Ancestral communities integrated its vegetative parts, roots, and calcified nutlets into medical practice across Europe, Western Asia, North America, and the Indian subcontinent [5, 6]. Ethnomedicinal records detail its administration for treating urogenital gravel, acute dysuria, digestive disorders, inflammatory cutaneous lesions, burns, measles, and smallpox, as well as an oral contraceptive and mild sedative [6, 7, 8].

The pharmacological actions of *L. officinale* arise from diverse specialized chemical constituents concentrated differentially across tissue types [9]. Subterranean root tissues serve as the primary site for synthesizing lipophilic hydroxynaphthoquinones, primarily shikonin and its acyl esters, which impart characteristic deep purple and crimson pigmentation [10, 11]. Beyond their pigmentary utility, these naphthoquinones display antimicrobial, wound-healing, antineoplastic, and anti-inflammatory properties [12, 13]. Aerial leaves and flowering stems contain rich reserves of phenolic compounds, predominantly rosmarinic acid, lithospermic acid, chlorogenic acid, and flavonoid glycosides like rutin and luteolin derivatives [9, 14, 15, 16]. The seeds possess mineralized endocarps containing elevated concentrations of calcium carbonate and silica, surrounding embryos rich in octadecatetraenoic, γ-linolenic, and stearidonic polyunsaturated fatty acids [17]. However, therapeutic deployment must contend with the presence of pyrrolizidine alkaloids, structurally characterized by 1,2-unsaturated necine bases that form reactive, hepatotoxic pyrroles through mammalian hepatic metabolism [7, 18, 19].

Modern drug discovery utilizes pharmaphylogeny to evaluate medicinal plants by systematically linking evolutionary lineages, phylogenetic grouping, chemical diversity, and documented pharmacological performance [3, 20]. *L. officinale* belongs to the tribe Lithospermeae, an assemblage containing genera such as *Arnebia*, *Onosma*, *Echium*, and *Alkanna*, which share the specialized biochemical capacity to generate alkannin-shikonin derivatives and related polyphenols [3, 20, 21]. Similarly, difficulties in

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domesticating slow-growing root systems and low metabolite yields have shifted focus toward plant-microbe interactions. Fungal endophytes colonizing the root cortical spaces of *L. officinale* have emerged as alternative producers of host-mimicking secondary metabolites, offering biotechnological solutions for chemical extraction without depleting wild plant populations. A critical, evidence-based integration of the historical use, chemical constituents, molecular biology, and pharmacological profile of *L. officinale* provides clear directions for clinical translation and safety governance.

Figure 1. Leaves and Flowers of *L. officinale*

The collection of data detailing historical findings, chemical structures, and biological actions was performed through a literature collection across electronic repositories, including PubMed and Google Scholar, covering literature ranging from 1952 to 2023. Specific retrieval queries included *L. officinale* L., *L. officinale* L. compounds, *L. officinale* L. phytochemicals, *L. officinale* L. pharmacological properties, and *L. officinale* L. traditional uses. Studies were considered eligible when they detailed empirical data regarding ethnobotanical applications, isolated secondary metabolites, qualitative and quantitative chromatography, and *in vitro* or *in vivo* biological evaluation. Reference lists of retrieved publications and authoritative historical botanical books were manually inspected to secure additional relevant literature. Table 1 summarizes the systematic search metrics, detailing the publication yields obtained across primary databases for each defined query string.

Table 1. Electronic database retrieval yields for *Lithospermum officinale* literature

Search Query	Google Scholar Records	PubMed Records	Scope
L. officinale L.	13,100	48	Broad botanical, agronomic, and biological indexing
L. officinale L. traditional uses	4,910	—	Ethnobotanical, folk medicinal, and regional documentation
L. officinale L. compounds	3,210	3	Isolation, fractionation, and structure elucidation
L. officinale L. pharmacological	2,840	6	*In vitro*, *in vivo*, and mechanistic biological assays
L. officinale L. phytochemicals	1,560	2	Metabolomic profiles, quantitative assays, and screening

2. Distribution and Characterization of *Lithospermum officinale*

2.1. Taxonomy and Distribution

Lithospermum officinale L. is classified within the family Boraginaceae, which encompasses approximately 135 genera and 2,600 species distributed across temperate and tropical zones worldwide [3, 4]. Within this family, the genus *Lithospermum* includes 50 to 60 species primarily adapted to temperate climates [4, 20]. *L. officinale* is an herbaceous perennial indigenous to temperate regions of Eurasia [4]. Anthropogenic dissemination and naturalization have expanded its presence into North America, specifically the United States, as well as temperate regions of South America, including Argentina and Colombia [4]. The plant thrives in well-drained calcareous and base-rich soils, limestone outcrops, forest margins, open scrublands, and abandoned fields.

2.2. Morphology

Lithospermum officinale is an erect perennial reaching up to 90 cm in height. The aerial stem is prominently branched in its upper portions and features two distinct types of pubescence: closely appressed, antrorse hairs and outward-directed, spreading hairs. These hairs reach up to 1.5 mm in length and originate from characteristically swollen, tuberculate bases.

Cauline leaves are broadly lanceolate, measuring approximately 60 to 70 mm in length and 10 to 16 mm in width. The leaf surfaces are densely covered with antrorse hairs reaching up to 1.9 mm long, which also arise from swollen bases, giving the foliage a rough, scabrous texture.

The inflorescence is organized into racemose cymes. Flowers are bracteate or subsessile, borne on pubescent pedicels that elongate up to 4 mm during fructification. The floral bracts are leafy in appearance but smaller than the cauline leaves. The calyx measures 5 to 6 mm in length, possesses antrorse hairs, features linear lobes, and elongates slightly as fruit develops. The corolla is white or cream-colored, consisting of a basal tube approximately 4 mm long capped by spreading, oval to blunt lobes with slightly wavy, scalloped margins, forming a corolla limb measuring 3.5 to 4 mm in width. The throat of the corolla tube is furnished with five small, sac-like hairy pouches. The anthers are oval, roughly 1 mm long with pointed tips, borne on very short filaments, and positioned within the tube alternating directly below the pouches. The style measures approximately 1.7 mm in length and terminates in a slightly rounded stigma.

2.3. Seed Characteristics

Fructification yields four erect, indehiscent nutlets. The nutlets measure 3 to 4 mm in length, displaying an ovoid geometry with an ivory to pale white, smooth, and shiny porcelain-like surface. The hard pericarp contains high concentrations of inorganic matter, where ash accounts for approximately 30% of total dry seed mass [9, 17]. Analytical quantification confirms that this mineralized matrix is composed predominantly of calcium carbonate (accounting for 68.2% of mineral salts or 59% of seed ash as calcium oxide) and silica (comprising 19.39% of mineral matter or 27% of seed ash), alongside smaller fractions of potassium, magnesium, phosphorus, nitrogen, and iron oxides [9, 17]. This structural composition protects the inner embryo and supports long-term seed dormancy [6].

3. Ethnomedicinal and Historical Uses

3.1. European Traditional Healthcare Systems

The therapeutic use of *L. officinale* across Europe spans from prehistory into early medical practice [6]. Archaeological excavations at prehistoric sites in Poland, dating between 1750 and 1600 B.C., identified nutlets of *L. officinale* employed as antiseptic agents and integrated into folk medicinal preparations [6]. Across classical European medicine, the hard nutlets were administered for urinary complaints [6, 8]. Following the Doctrine of Signatures, historical practitioners reasoned that the calcified, stony seeds possessed the capacity to dissolve and expel kidney stones and bladder gravel [7, 8]. Decoctions of the seeds and aerial parts served as diuretics, lithotriptics, and demulcents for urinary tract inflammation, strangury, and gout [7, 9]. In Spain, preparations derived from the leaves were utilized as sedative remedies to calm nervous agitation [5, 9].

3.2. Asian Traditional Formulations

Across Traditional Chinese Medicine and regional Asian pharmacopeias, *L. officinale* has been used to manage urogenital conditions, relieve internal spasms, clear toxic heat, and regulate fluid retention [5, 9]. Beyond medicinal applications, archaeological evidence from the Yanghai Tombs in Xinjiang, China, revealed nutlets of *L. officinale* affixed to wooden tubs, illustrating their use as decorative and cultural artifacts during the first millennium B.C.

In traditional healthcare systems of India, distinct plant parts were applied for specific therapeutic outcomes [5, 22]. The leaves were administered as sedative agents, whereas the seeds were used for their diuretic and lithotriptic properties [5, 22]. Syrups and decoctions prepared from the roots and twigs were given to soothe eruptive fevers, such as smallpox and measles, while herbal teas prepared from the roots and stems were used to alleviate cutaneous itching and systemic eruptive diseases [5, 22]. Externally, smooth seeds were placed beneath the eyelids to facilitate the mechanical extraction of foreign particles from the eye [5, 9]. Subterranean roots were also harvested as natural dyes for coloring textiles and preparing cosmetic pigments. Table 2 outlines the documented traditional medicinal uses of *Lithospermum officinale*, detailing the plant parts employed, geographical regions of practice, and primary therapeutic indications.

Table 2. Ethnomedicinal applications and traditional indications of *Lithospermum officinale*

Region	Plant Part Used	Preparation	Traditional Therapeutic Applications
Poland (Central Europe)	Mature nutlets (fruits)	Decoctions, powdered preparations	Antiseptic application, topical wound management, folk medicinal formulations
Classical Europe / Mediterranean	Aerial parts, seeds, and nutlets	Aqueous infusions, wine-macerated decoctions	Litholytic, diuretic, relief of urinary strangury, gravel expulsion, and anti-gout therapy
China (East Asia)	Fruits (nutlets) and roots	Decoctions, aqueous infusions	Spasmolytic relief, urogenital disorders, clearing toxic heat, and decorative burial artifacts
India (South Asia)	Leaves	Aqueous infusions, crushed leaf extracts	Sedative, calming nervous agitation
India (South Asia)	Seeds	Macerated or powdered preparations	Diuretic, lithotriptic, and removal of foreign particles from the eye
India (South Asia)	Roots and twigs	Syrups, decoctions, and herbal teas	Eruptive fevers (measles, smallpox) and alleviation of cutaneous pruritus
North America (Indigenous cultures)	Roots	Cold-water extracts and decoctions	Antidiarrheal therapy and systemic oral fertility regulation (antiovulatory use)

3.3. Indigenous American Applications

Ethnomedicinal records from North America document related traditional applications among indigenous populations, particularly with the related species *Lithospermum ruderale* [7, 9]. Indigenous communities consumed root preparations to control acute diarrhea [7]. Cold-water infusions of the roots were prepared by tribes in Nevada as oral contraceptives to suppress ovulation and regulate fertility, which subsequently motivated scientific investigations into the endocrine-modulating properties of the genus [7, 9].

4. Phytochemical Constituents

4.1. Lipophilic Naphthoquinones

Subterranean root tissues of *L. officinale* synthesize lipophilic 1,4-naphthoquinones, primarily shikonin, alkannin, and their esterified derivatives [9, 13]. Shikonin features an isohexenyl side chain attached to a 5,8-dihydroxy-1,4-naphthoquinone core [10, 13]. Quantitative chromatographic profiling reveals that dried roots contain shikonin at concentrations of 0.079 ± 0.002 mg/g, alongside acetylshikonin and related derivatives [9]. The biosynthesized naphthoquinones display enantiomeric divergence: shikonin possesses an (R)-chiral configuration, whereas alkannin possesses an (S)-chiral configuration [10, 13]. These compounds confer purple-red pigmentation to the root periderm and function as antimicrobial and defensive secondary metabolites [10, 13].

4.2. Phenolic Acids and Polyphenols

Polyphenolic acids constitute a prominent polar fraction in the shoots and roots of *L. officinale* [9, 14]. Quantitative analyses show that rosmarinic acid accumulates at 1.2 ± 0.1 mg/g dry weight in shoots and reaches 1.8±0.31 mg/g in roots [9]. Lithospermic acid, a complex caffeic acid tetramer, was historically isolated from *L. officinale* and characterized as a central bioactive constituent [14]. Additional phenolic acids identified across the vegetative organs include chlorogenic acid (1.032±0.06 mg/g in shoots), hydrocaffeic acid (0.215±0.017 mg/g in shoots; 0.131±0.015 mg/g in roots), and p-hydroxybenzoic acid (0.174±0.003 mg/g in shoots), along with gallotannins and catechin-type condensed tannins [9, 16].

4.3. Flavonoids and Flavonol Glycosides

Flavonoid derivatives are present within the leaves, stems, and inflorescences [9, 15]. The flavonol glycoside rutin accumulates at concentrations of 0.754±0.303 mg/g dry matter in shoots [9, 23]. Luteolin-7β-glucuronide has also been isolated from the aerial tissues, contributing to antioxidant, radical-scavenging, and anti-inflammatory activities [9, 15].

Table 3 lists out the major phytochemical constituents identified in *Lithospermum officinale*, specifying their chemical classes, localized plant organs, and reported quantitative yields.

Table 3. Phytochemical constituents isolated from vegetative and reproductive organs of *Lithospermum officinale*

Compound Class	Specific Chemical Entity	Plant Organ / Tissue	Reported Quantitative Concentration / Content
Naphthoquinones	Shikonin	Subterranean roots	0.079±0.002 mg/g dry weight
Naphthoquinones	Acetylshikonin, alkannin derivatives	Root periderm	Qualitative identification
Phenolic acids	Rosmarinic acid	Aerial shoots; roots	1.20±0.10 mg/g (shoots); 1.80±0.31 mg/g (roots)
Phenolic acids	Lithospermic acid	Whole plant, roots	Characteristic caffeic acid tetramer
Phenolic acids	Chlorogenic acid	Aerial shoots	1.032±0.060 mg/g dry weight
Phenolic acids	Hydrocaffeic acid	Shoots; roots	0.215±0.017 mg/g (shoots); 0.131±0.015 mg/g (roots)
Phenolic acids	p-Hydroxybenzoic acid	Aerial shoots	0.174±0.003 mg/g dry weight
Flavonoids	Rutin	Aerial shoots	0.754±0.303 mg/g dry weight
Flavonoids	Luteolin-7 β -glucuronide	Leaves, aerial parts	Bioactive glucuronide fraction
Pyrrolizidine alkaloids	Lithosenine, acetyllithosenine	Roots, aerial vegetative parts	Trace retronecine-type diesters
Purine derivatives	Allantoin	Shoots; roots	2.36 ± 0.88 mg/g (shoots); 0.81±0.36 mg/g (roots)
Polyunsaturated fatty acids	Stearidonic acid, γ -linolenic acid	Seed lipid matrix	Constitutes major portion of 17–20% seed fatty oil
Inorganic salts	Calcium carbonate, silica	Mineralized seed pericarp	68.2% $CaCO_3$ and 19.39% SiO_2 of total mineral matter

4.4. Pyrrolizidine Alkaloids

Like many members of the Boraginaceae family, *L. officinale* produces 1,2-dehydropyrrolizidine alkaloids [11, 18]. Phytochemical investigations have isolated O^7-(3-hydroxy-3-methylbutanoyl)-O^9-(-)-hydroxyviridiflorylretronecine (lithosenine) and its acetylated derivative, acetyllithosenine [18]. Other related necine-base derivatives identified within the plant include lycopsamine and echimidine [7, 18]. These alkaloids contain a 1,2-unsaturated necine nucleus capable of undergoing metabolic activation into reactive pyrroles, which represents the primary toxicological risk of the species [7].

4.5. Lipids and Seed Fatty Acids

The seeds contain approximately 17% to 20% fatty oil rich in polyunsaturated fatty acids [9, 17]. The lipid fraction comprises neutral triacylglycerols, phosphatides (1.3%), phytoglucolipids, monophosphoinositides, phosphatidylethanolamine, phosphatidylcholine, cerebrosides, and sterols such as β-sitosterol and Δ^5-avenasterol [9, 17]. Fatty acid analysis shows significant proportions of palmitic, stearic, oleic, linolenic, hexadecadienoic, hydroxypentacosenoic, hydroxyeicosatrienoic, and tetraenoic acids [9, 17]. The oil contains notable concentrations of stearidonic acid (SDA, 18:4n-3), γ-linolenic acid (GLA, 18:3n-6), and 6,9,12,15-n-octadecatetraenoic acid, along with fructans and fat-soluble vitamin E [9, 17].

4.6. Cyanogenic Glycosides and Other Bioactive Metabolites

The roots yield a unique cyanogenic glycoside identified as 6- O - β -D-glucopyranosyl-1-cyanomethylene-4,5-dihydroxy-2-cyclohexene [9, 24]. Shoot and root tissues also contain high concentrations of allantoin, accumulating at 2.36±0.88 mg/g dry matter in shoots and 0.81±0.36 mg/g in subterranean roots, which contributes to tissue-repair and keratolytic properties [9].

5. Pharmacological Activities and Biological Mechanisms

5.1. Neuroprotective and Anti-Inflammatory Activities

The anti-inflammatory and neuroprotective potential of *L. officinale* has been established using cell culture models [25]. A methanolic extract obtained from 17-day-old unorganized callus tissue (LoE) was evaluated on primary rat microglial cells stimulated with lipopolysaccharide (LPS) [25]. At a concentration of 0.8 mg/mL, LoE significantly downregulated the transcription of key pro-inflammatory genes, including inducible nitric oxide synthase (*Nos2*), cyclooxygenase-2 (*Cox-2*), and tumor necrosis factor-alpha

(*Tnf-α*) [25]. Treatment also suppressed the secretion of TNF-α and interleukin-1 beta (IL-1 β) cytokines into culture media, displaying greater inhibitory activity than commercial reference formulations [25]. This neuroprotective action is largely attributed to rosmarinic acid, which suppresses nuclear factor kappa B (NF-κB) nuclear translocation and attenuates microglial-mediated neurotoxicity [16, 25].

5.2. Wound Repair and Burn Healing

The wound-healing capacity of *L. officinale* was evaluated *in vivo* using a standardized rat thermal burn model [12]. Topical administration of an ointment containing *L. officinale* was evaluated alongside 1% silver sulfadiazine (SSD) and an herbal commercial formulation (Alpha ointment) [12]. Histopathological examination showed that *L. officinale* treatment reduced inflammatory-cell infiltration and macrophage accumulation within burn tissue [12]. The extract stimulated rapid re-epithelialization, accelerated neovascularization, and promoted organized granulation tissue formation [12]. These regenerative responses were comparable, and in several parameters superior, to those seen with silver sulfadiazine, which often causes local tissue toxicity [12]. This wound-healing efficacy is driven by the synergistic actions of shikonin, which stimulates microvascular angiogenesis and fibroblast migration, and allantoin, which accelerates epidermal cellular regeneration and debridement [7, 12, 13].

5.3. Antithyrotropic and Endocrine Effects

Freeze-dried aqueous extracts of *L. officinale* (Lith. off. FDE) display marked antithyrotropic properties in animal models [7]. In rats, co-administration of Lith. off. FDE with exogenous thyroid-stimulating hormone (TSH) prevented the TSH-induced increase in thyroidal endocytotic colloid droplet formation, inhibiting thyroid hormone release [7]. When administered alone, the extract decreased endogenous serum TSH levels, lowered thyroidal secretion rates, and reduced circulating concentrations of thyroxine (T_4) and triiodothyronine (T_3) [7]. The inhibitory effect of the extract was more rapid and sustained than that of potassium iodide (KI) [7]. In thyroidectomized rats maintained on exogenous thyroxine, Lith. off. FDE inhibited the peripheral monodeiodination of T_4 into biologically active T_3 [7]. These antigonadotropic and antithyrotropic activities are linked to oxidized polyphenolic constituents, particularly lithospermic acid, which modify peptide hormone conformations and disrupt hormone-receptor binding [7, 14]. Table 4 highlights the experimentally verified pharmacological activities of *Lithospermum officinale* extracts and pure constituents, summarizing their experimental models and observed biological responses.

Table 4. Experimentally proven pharmacological activities of *Lithospermum officinale*

Pharmacological Activity	Extract Type / Compound	Experimental Assay / Test Model	Biological Mechanisms and Key Outcomes
Anti-neuroinflammatory / Neuroprotective	Methanolic callus extract (LoE)	LPS-activated primary rat microglial cell cultures	Downregulation of *Nos2*, *Cox-2*, and *Tnf-α* transcripts; inhibition of secreted TNF-α and IL-1β cytokines
Wound and Thermal Burn Repair	Crude extract topical ointment	Deep contact thermal burn model in Sprague-Dawley rats	Reduction of PMN leukocytes and macrophages; stimulation of neovascularization, fibroblast proliferation, and rapid re-epithelialization
Antithyrotropic / Endocrine Modulation	Freeze-dried aqueous extract (Lith. off. FDE)	In vivo intact and thyroidectomized Wistar rats	Blockade of TSH-induced thyroid endocytosis; reduction of circulating T_3 and T_4; inhibition of peripheral conversion of T_4 to T_3
Antimicrobial	Shikonin, alkannin, and lipophilic root extracts	In vitro broth microdilution assays	Intercalation into bacterial cytoplasmic membranes, loss of membrane potential, and cellular lysis in Gram-positive bacteria
Antineoplastic / Cytotoxic	Pure shikonin and rosmarinic acid	Human malignant cell lines (A549, HepG2, HeLa, MDA-MB-231)	PKM2 enzymatic inhibition; intracellular ROS overgeneration; caspase-mediated apoptosis and induction of RIPK1/RIPK3-dependent necroptosis

5.4. Antimicrobial and Antiparasitic Actions

L. officinale extracts and isolated naphthoquinones display antimicrobial activity against pathogenic bacterial and fungal species [9, 13]. Shikonin, alkannin, and their lipophilic esters disrupt bacterial cytoplasmic membranes, alter membrane potential, and inhibit respiratory dehydrogenases in Gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus subtilis* [10, 13]. Gram-negative organisms show greater resistance due to their protective outer lipopolysaccharide layer [10]. In antiparasitic assays, naphthoquinone fractions cause mitochondrial membrane depolarization, reactive oxygen species generation, and cell death in protozoal parasites [13].

5.5. Antineoplastic Mechanisms

The anticancer properties of *L. officinale* compounds, particularly shikonin and alkannin, involve multiple cellular mechanisms [13, 26]. Shikonin selectively inhibits the glycolytic enzyme pyruvate kinase M2 (PKM2), suppressing aerobic glycolysis in malignant cells [13]. Intracellular redox cycling of the naphthoquinone core produces excessive intracellular reactive oxygen species (ROS), which promotes mitochondrial permeability transition pore opening, releases cytochrome *c*, and activates executioner caspases to trigger apoptosis [13, 26]. In apoptosis-resistant tumors, shikonin bypasses apoptotic blocks by activating receptor-interacting protein kinases (RIPK1 and RIPK3), driving programmed necroptosis [13]. Similarly, rosmarinic acid suppresses cancer cell invasion and migration by inhibiting the PI3K/Akt/mTOR pathway and downregulating FOXM1 transcriptional activity [16]. Figure 2 shows the multi-target molecular mechanisms of L. officinale bioactive metabolites across microglial neuroprotection, dermal burn restoration, and metabolic/glycolytic inhibition.

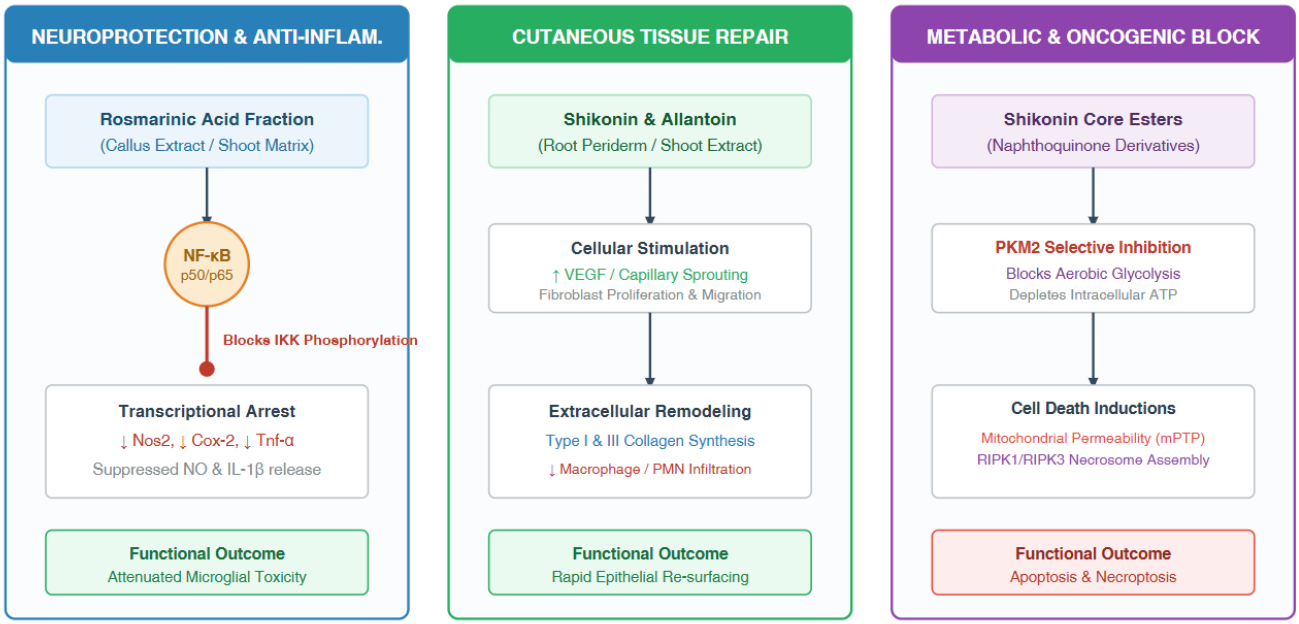

Figure 2. Pharmacological Mode of Action of *L. officinale*

6. Adverse reactions and Safety

The major safety challenge associated with *L. officinale* is the presence of 1,2-dehydropyrrolizidine alkaloids, including lithosenine, lycopsamine, and echimidine [7, 18]. When ingested, these unsaturated alkaloids undergo bioactivation by hepatic cytochrome P450 monooxygenases (predominantly CYP3A4) into electrophilic dehydropyrrolic esters [7]. These reactive intermediates covalently bind to nucleophilic cellular proteins and genomic DNA, forming stable adducts that cause hepatic sinusoidal obstruction syndrome (HSOS), liver cirrhosis, and genotoxicity [7].

Although cutaneous absorption of pyrrolizidine alkaloids through intact human skin appears low, oral consumption of crude preparations carries definite hepatotoxic risks [7]. Regulatory authorities, including the European Medicines Agency (EMA), enforce strict daily exposure thresholds (less than 0.35 to 1.0 μg per day) for herbal products containing unsaturated pyrrolizidine alkaloids [7]. Consequently, developing clinical products from *L. officinale* requires targeted extraction methods, such as solid-phase strong cation-exchange resins or the use of PA-free cell cultures, to eliminate toxic alkaloids while retaining therapeutic naphthoquinones and phenolics [19].

7. Evolutionary Pharmaphylogeny of Lithospermeae

The family of Lithospermeae (subfamily Boraginoideae, Boraginaceae) is primarily distributed across western Eurasia and the Mediterranean basin, having diversified during the Miocene epoch [3, 20]. Members of this tribe adapted to arid and semi-arid conditions by evolving specialized secondary metabolic pathways, notably synthesizing alkannin and shikonin naphthoquinones [3, 10, 21].

Based on molecular phylogeny, geographical distribution, phytochemical profiles, and ethnomedicinal applications, the tribe Lithospermeae can be categorized into three major groups [3, 20, 21]:

- Group 1: Comprises *Onosma* and *Maharanga* [3, 21]. These taxa are rich in alkannin, shikonin, flavonoids, and phenolic acids, and are traditionally used to treat wounds, burns, respiratory ailments, and inflammatory conditions [21].
- Group 2: Comprises *Echium* and *Lobostemon* [3]. These genera synthesize pyrrolizidine alkaloids, phenolic acids, and polyunsaturated seed fatty acids [3]. They are utilized in traditional medicine for wound healing, ulcers, anxiety, and inflammatory disorders [3].
- Group 3: Comprises *Alkanna*, *Arnebia*, *Stenosolenium*, *Lithodora*, *Lithospermum*, *Buglossoides*, and *Glandora* [3, 20]. Species in this lineage are defined by high concentrations of naphthoquinones, rosmarinic acid, and lithospermic acid [3, 10, 14]. They are historically applied to treat burns, eruptive skin disorders, circulatory conditions, and urogenital gravel [5, 12, 21].

Table 5 categorizes the primary lineages of the tribe Lithospermeae, contrasting their defining chemical constituents, traditional applications, and showed pharmacological activities.

Table 5. Pharmaphylogenetic classification and chemotaxonomic attributes within the tribe Lithospermeae

Lineage Classification	Genus	Characteristic Phytochemical Markers	Traditional Ethnomedicinal Applications	Pharmacological Activities
Group 1	*Onosma*, *Maharanga*	Alkannin/shikonin enantiomers, flavones, rosmarinic acid dimers	Cutaneous burns, pulmonary infections, wound management, and swelling	Wound healing, anti-inflammatory, antimicrobial, and analgesic activities
Group 2	*Echium*, *Lobostemon*	Macrocyclic pyrrolizidine alkaloids, phenolic acids, stearidonic acid	Ulcers, cutaneous lesions, nervous excitation, and inflammatory disorders	Anti-inflammatory, antimicrobial, anxiolytic, and anti-HIV properties
Group 3	*Lithospermum*, *Arnebia*, *Alkanna*, *Stenosolenium*, *Lithodora*, *Buglossoides*, *Glandora*	Enantiomeric naphthoquinones (shikonin and alkannin esters), lithospermic acid, chlorogenic acid	Urolithiasis, acute diarrhea, smallpox, measles, joint pain, cough, and detoxification	Wound healing, antineoplastic, antithyrotropic, antioxidant, antithrombotic, and antimicrobial effects

Within Group 3, *Alkanna* predominantly synthesizes (S)-alkannin derivatives, while *Lithospermum* and *Arnebia* accumulate (R)-shikonin enantiomers, indicating enzymatic cleavage in their biosynthetic pathways [10, 21]. *Lithodora* remains relatively uncharacterized chemically, whereas *Stenosolenium* has traditional applications in treating joint pain and bleeding. Analyzing these evolutionary relationships provides a framework for predicting chemical constituents in underexplored relatives and identifying alternative botanical sources of bioactive compounds [3, 20]. Figure 3 shows the systematic tri-lineage divergence of the tribe Lithospermeae, outlining the chemotaxonomic distribution of specialized naphthoquinone enantiomers and polyphenols

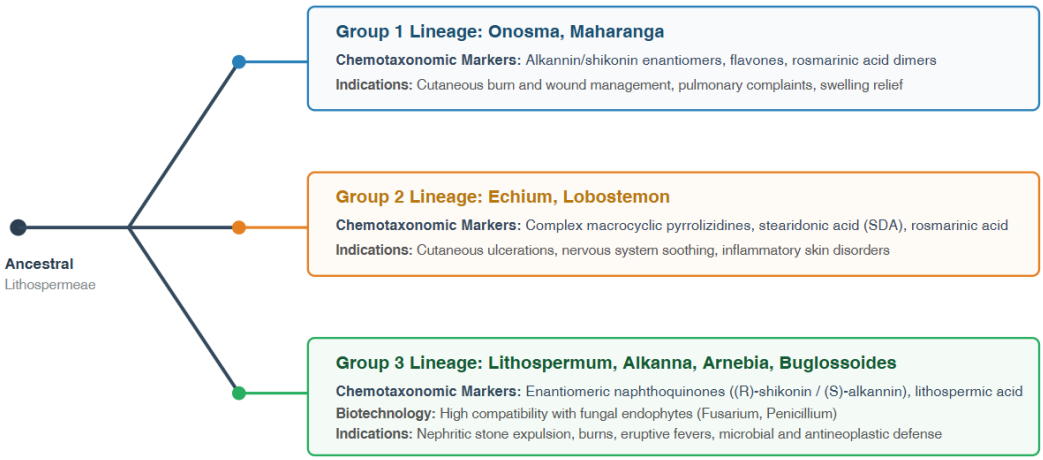

Figure 3. Tri-Lineage Pharmaphylogenetic Cladogram of the Tribe Lithospermeae

8. Endophytic Fungi as Alternative Producers of Secondary Metabolites

Fungal endophytes colonize the internal root and shoot tissues of *L. officinale* without causing visible signs of disease. These mutualistic endophytes produce bioactive secondary metabolites, including compounds identical or structurally related to host-plant secondary metabolites. Microbiological isolation from *L. officinale* roots, followed by internal transcribed spacer (ITS) rDNA sequencing, has identified several endophytic fungi:

- *Fusarium solani* and related *Fusarium* species
- *Penicillium citrinum*
- *Aspergillus niger*
- *Trichoderma viride*

Fermentation cultures of these endophytic fungi, particularly strains within the *Fusarium* complex, possess the enzymatic machinery to synthesize shikonin and related naphthoquinones in axenic liquid media. High-performance liquid chromatography (HPLC) confirms that fungal shikonin matches the retention times and spectral characteristics of plant-derived reference standards. Liquid fermentation of endophytic fungi offers a controlled, sustainable platform for producing pharmaceutical-grade shikonin and polyphenols, circumventing the slow growth cycles of field-cultivated plants and protecting wild populations from overexploitation [10].

9. Conclusion

Lithospermum officinale possesses an extensive history of traditional medicinal applications and a diverse phytochemical profile. Its specialized metabolites including lipophilic shikonin derivatives, rosmarinic, lithospermic, and chlorogenic acids, along with seed-derived stearidonic and γ-linolenic acids provide a biological foundation for its reported antioxidant, anti-inflammatory, wound-healing, antimicrobial, and antithyrotropic activities. The presence of hepatotoxic 1,2-dehydropyrrolizidine alkaloids emphasizes the necessity of developing standardized detoxification protocols and adopting chromatographic quality control. Applying pharmaphylogenetic principles across the tribe Lithospermeae, combined with bioprospecting mutualistic fungal endophytes, provides a sustainable strategy for metabolite production.

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