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REVIEW

A Natural Compound Methylnissolin: Physicochemical Properties, Pharmacological Activities, Pharmacokinetics and Resource Development

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Abstract: Methylnissolin (also known as Astrapterocarpan) is an isoflavonoid compound featuring a pterocarpan core structure. To date, leguminous plants of the genus *Astragalus* remain the exclusive natural source of Methylnissolin and its glycoside derivative, Methylnissolin-3-O-glucoside. Upon oral administration, Methylnissolin and its glycosides enter systemic circulation and modulate signaling pathways such as RIPK2/ASK1, PI3K/AKT, IκB/NF-κB, MAPK, and Nrf2/HO-1. Their pharmacological activities span anti-inflammatory, antioxidant, glucose-lipid metabolism regulation, and antitumor effects, underscoring their broad potential for drug development. This review comprehensively evaluates the physicochemical properties, pharmacological activities, mechanisms of action, pharmacokinetic characteristics, and toxicological profile of Methylnissolin and its glycoside derivatives. Notably, we systematically elucidate the metabolic fate of methylnissolin, identifying hydroxylation, demethylation, dimerization, hydration, and dehydrogenation as predominant biotransformation pathways. Furthermore, the influence of factors such as plant variety, geographical origin, and processing methods on Methylnissolin and its glycoside content in *Astragalus membranaceus* is analyzed, providing crucial insights for drug development and resource utilization.

Keywords: methylnissolin, methylnissolin-3-O-glucoside, astrapterocarpan, astragalus mongholicus, pharmacokinetics, pharmacological activity

Introduction

Astragalus mongholicus, a leguminous plant, is widely used internationally and has pharmacological effects such as anti-fibrosis,[1–3] anti-tumor metastasis,[4–6] and improvement of diabetic nephropathy[7–9] and non-alcoholic fatty liver disease.[10–12] In recent years, the importance of natural products in drug research and development has become increasingly prominent. Various active components isolated and identified from *Astragalus mongholicus* provide potential lead compounds for new drugs.

In *Astragalus mongholicus*, polysaccharides and saponins constitute the predominant components; however, their pharmaceutical potential is constrained by intrinsic limitations. Polysaccharides, despite their high abundance, exhibit poorly defined structural configurations and low oral bioavailability due to their large molecular size (eg, Astragalus polysaccharides: 1334 kDa) and susceptibility to gastrointestinal degradation.[13] Similarly, saponins such as astragalosides encounter absorption challenges attributed to limited membrane permeability and extensive first-pass metabolism. For instance, the absolute bioavailability of Astragaloside IV in rats is merely 2.2%.[14] In contrast, flavonoid-derived components, particularly those detected as prototype compounds in systemic circulation, hold unique translational

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value. According to the database of constituents absorbed into the blood of Traditional Chinese Medicine,[15] 51 prototype compounds from *Astragalus mongholicus* have been identified in blood, with flavonoid derivatives representing the largest proportion (29.41%, 15/51). Notably, Methylnissolin, a flavonoid derivative characterized by a pterocarpan core structure, exhibits exceptional pharmaceutical potential due to its favorable pharmacokinetic properties and marked pharmacological activity. It is absorbed into the bloodstream and excreted predominantly in its unmetabolized form,[16,17] demonstrating superior oral bioavailability (64.26%).[18] Furthermore, its drug-likeness score (0.42, based on Lipinski's rule of five) positions methylnissolin as a prioritized candidate for further development.

Pterocarpans are a class of dihydroisoflavonoid secondary metabolites with phytoalexin properties.[19,20] In recent years, it has also been reported to have effects similar to those of *Astragalus mongholicus*, including inhibiting cancer cells,[21–23] suppressing inflammation,[24–27] and improving blood sugar[20,28] and lipid[20,28] levels. The structural feature of Pterocarpans is a tetracyclic structure composed of a benzofuran-benzopyran, with two chiral centers at positions C-6a and C-11a determining the molecule's stereochemistry, as shown in Figure 1. Although computational studies suggest that the energy of C/D ring trans isomers is much lower, only compounds with a C/D ring cis structure exist in nature.[29] Studies have shown that Methylnissolin and its derivative Methylnissolin-3-O-glucoside are present in *Astragalus mongholicus*.[30–34] Compared to chemically synthesized drugs, natural products often have unique mechanisms of action. Through long-term natural selection processes, they have formed complex and subtle interactions with biological systems, potentially offering unique advantages in disease treatment.

This study aims to comprehensively review the research status of Methylnissolin and its glycoside compounds in terms of physicochemical properties, biological activity, pharmacokinetics, and influencing factors, providing a reference for subsequent in-depth research and development.

Physicochemical Properties

Methylnissolin, also known as Astrapterocarpan, has a molecular formula of $C_{17}H_{16}O_5$, a molecular weight of 300.30 g/mol, and a density of 1.3±0.1 g/cm³,[35] as shown in Table 1. Due to the additional glucose group, Methylnissolin-3-O-glucoside has a larger molecular weight (300.30 vs 462.4 g/mol) and a higher density (1.5±0.1 vs 1.3±0.1 g/cm³). The boiling point and evaporation enthalpy also show higher values for Methylnissolin-3-O-glucoside, which may be due to the stronger intermolecular forces resulting from its larger molecular weight and more polar functional groups. This indicates that Methylnissolin-3-O-glucoside is more stable at high temperatures, possibly suitable for high-temperature extraction methods. The ACD/LogP value of Methylnissolin is 2.45, significantly higher than that of Methylnissolin-3-O-glucoside, which is 0.41, indicating that Methylnissolin has stronger lipophilicity. Additionally, due to its higher LogP and LogD, Methylnissolin may have advantages in absorption and distribution within the body. Methylnissolin-

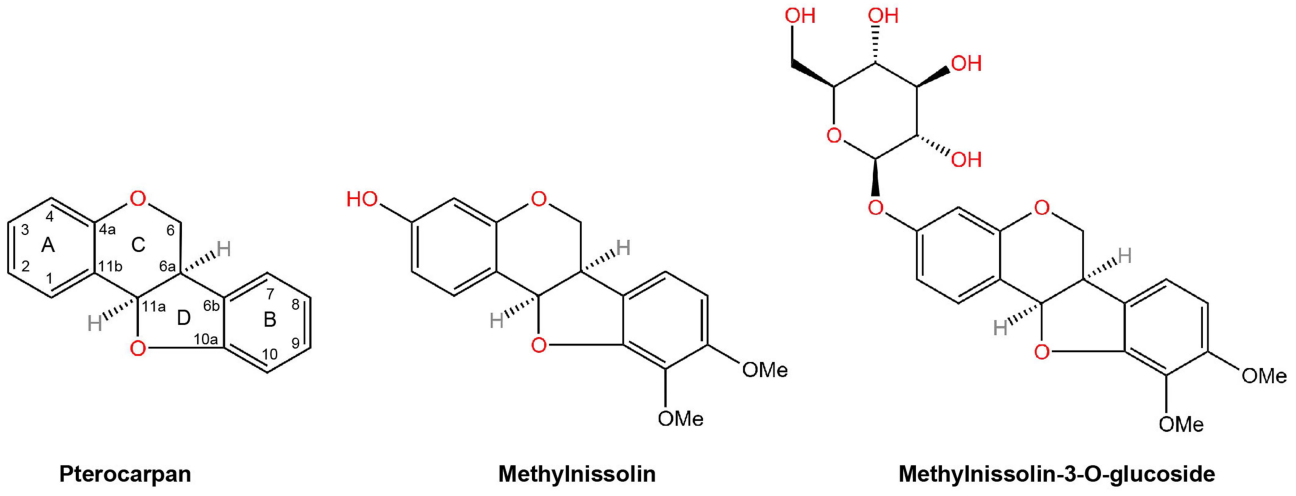

Figure 1 The chemical structures of pterocarpan compounds methylnissolin and methylnissolin-3-O-glucoside.

Table 1 Physicochemical Properties of Methylnissolin and Its Glycoside

Name	Methylnissolin	Methylnissolin-3-O-glucoside
Molecular Formula	$C_{17}H_{16}O_5$	$C_{23}H_{26}O_{10}$
Molecular Weight	300.30 g/mol	462.4 g/mol
Density	1.3±0.1 g/cm³	1.5±0.1 g/cm³
Boiling point	428.9±45.0 °C at 760 mmHg	646.3±55.0 °C at 760 mmHg
Vapour pressure	0.0±1.1 mmHg at 25°C	0.0±2.0 mmHg at 25°C
Enthalpy of vaporization	71.1±3.0 kJ/mol	100.2±3.0 kJ/mol
Flash point	213.2±28.7 °C	344.7±31.5 °C
Index of refraction	1.612	1.633
Molar refractivity	79.5±0.3 cm³	113.3±0.3 cm³
H bond acceptors	5	10
H bond donors	1	4
Freely rotating bonds	2	5
Rule of 5 violations	0	1 (violation: TPSA>131.6)
Polar surface area	57 Å ²	136 Å ²
Polarizability	31.5±0.5 10^{-24}cm³	44.9±0.5 10^{-24}cm³
Surface tension	51.5±3.0 dyne/cm	61.2±3.0 dyne/cm
Molar volume	228.8±3.0 cm³	317.5±3.0 cm³
ACD/LogP	2.45	0.41
ACD/LogD (pH 5.5)	2.81	0.66
ACD/BCF (pH 5.5)	80.4	1.88
ACD/KOC (pH 5.5)	804.23	54.73
ACD/LogD (pH 7.4)	2.81	0.66
ACD/BCF (pH 7.4)	79.69	1.88
ACD/KOC (pH 7.4)	797.13	54.73

3-O-glucoside has more hydrogen bond acceptors and donors, making it more soluble in polar environments such as water. However, its larger polar surface area (violating Lipinski's rule) may lead to easier breakdown in the digestive tract.[36,37] Therefore, Methylnissolin seems to show greater potential in oral drug development compared to Methylnissolin-3-O-glucoside.

Biological Activity

Anti-Inflammatory

The anti-inflammatory activity of Methylnissolin and its glycoside has been verified in models of adaptive immunity, metabolic inflammation, and infectious acute inflammation induced by Concanavalin A, adipose co-culture, and lipopolysaccharide (LPS). Concanavalin A is a plant lectin commonly used in vitro to study T cell-mediated immune responses.[38,39] Treatment with 30 μM Methylnissolin-3-O-glucoside for 48 hours can significantly inhibit T cell proliferation induced by Concanavalin A (2.5 μmol) in primary mouse spleen cells.[40] This effect is not due to cytotoxicity, as Methylnissolin-3-O-glucoside does not affect the activity of 3T3-L1 preadipocytes and RAW264.7 macrophages at concentrations up to 100 μM.[41] Under co-culture conditions of 3T3-L1 and RAW264.7 cells, Methylnissolin-3-O-glucoside significantly inhibits the production of pro-inflammatory factors IL-6 and MCP-1. Its mechanism of action may be related to the inhibition of COX-2 and iNOS expression.[41] LPS is a component of bacterial cell walls and is often used to simulate acute inflammatory responses caused by bacterial infections. In LPS-stimulated RAW 264.7 macrophages, methylnissolin and its glucoside inhibited the IκB/NF-κB inflammatory signaling pathway in a dose-dependent manner, with a minimum effective concentration of 3.3 μM. Mechanistically, methylnissolin suppressed the phosphorylation of IκB-α, thereby blocking nuclear translocation of the NF-κB p65 subunit. Nuclear accumulation of p65 activates transcription of pro-inflammatory genes (eg, IL-1β, IL-6, TNF-α) via direct promoter binding. This mechanism aligns with the observed downregulation of these cytokines at both mRNA and protein levels in vitro and in vivo, elucidating the anti-inflammatory efficacy of methylnissolin.[42] Additionally, Li

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et al extracted primary bone marrow-derived dendritic cells from mice and used LPS to induce IL-12 p40 production, evaluating the inhibitory effect of Methylnissolin.[43] IL-12 p40 can stimulate the activity of T cells and natural killer cells, which is crucial for antiviral and antitumor immune responses. It was found that Methylnissolin-3-O-glucoside at a concentration of 25 μM showed weak inhibitory effects on LPS-induced IL-12 p40 production, whereas Methylnissolin at the same concentration displayed moderate inhibitory effects.[43] This suggests that Methylnissolin shows more promising immunomodulatory activity than its glycoside, a result further confirmed by in vivo experiments. In an LPS/D-galactosamine-induced liver injury mouse model, prophylactic administration of Methylnissolin, but not Methylnissolin-3-O-glucoside, (20 mg/kg, intraperitoneal injection) for three consecutive days significantly improved liver pathological changes, liver function indicators (ALT, AST), inflammatory factors (IL-6, TNF-α), and the phosphorylation of hepatic P65 and IKB-α.[42]

Antioxidant

The antioxidant effect appears to be more pronounced in Methylnissolin-3-O-glucoside than in Methylnissolin. The initial report identified seven antioxidant substances, including Methylnissolin-3-O-glucoside, in the methanol:chloroform (3:17) eluate from *Astragalus mongholicus* and *Paeonia lactiflora Pall*.[44] Subsequently, in lung fibroblasts MRC-5 cells, it showed the strongest synergy in scavenging DPPH free radicals and reducing iron ions. To better understand the relationship between antioxidant capacity and Methylnissolin-3-O-glucoside content, researchers conducted three radical scavenging experiments and calculated the Pearson correlation coefficient.[45] The results showed a significant correlation between the content of Methylnissolin-3-O-glucoside and antioxidant capacity (DPPH=0.675, ABTS=0.670, FRAP=0.949). Mechanistically, Methylnissolin-3-O-glucoside was identified as a potential Nrf2 activator through ARE-dependent luciferase activity.[46] At 5 μM, the induction of the reporter gene was enhanced approximately twofold, while at 80 μM, it was enhanced 20-fold. Western blot and reverse transcription-polymerase chain reaction results revealed that Methylnissolin-3-O-glucoside could effectively induce the expression of AKT/Nrf2 and its downstream target genes HO-1 and NQO1 in EA.hy926 cells, with the induction showing a clear dose-dependent and time-dependent manner, being most significant at 80 μM.[46] Silencing with Nrf2 siRNA could reverse these beneficial effects of Methylnissolin-3-O-glucoside (see Figure 2). However, treatment with an AKT inhibitor suppressed the expression of Nrf2 and HO-1 only partially,[46] suggesting that Methylnissolin-3-O-glucoside might regulate the Nrf2/HO-1 pathway via multiple mechanisms. This multi-target regulation highlights the therapeutic potential of Methylnissolin-3-O-glucoside in oxidative stress-related pathologies. Specifically, chronic activation of the Nrf2/HO-1 axis could mitigate key pathological processes—such as inflammation, mitochondrial dysfunction, and tissue damage—in cardiovascular diseases (eg, atherosclerosis),[47] neurodegenerative disorders (eg, Alzheimer's and Parkinson's disease),[48,49] and diabetic complications.[8,50] These findings position Methylnissolin-3-O-glucoside as a promising candidate for developing nutraceutical or pharmacological agents aimed at restoring redox homeostasis.

Regulation of Glucose and Lipid Metabolism

Tang et al demonstrated that Methylnissolin exerts a dose-dependent inhibitory effect on high glucose-induced pathological proliferation of rat mesangial cells,[52] a key cellular event in diabetic kidney disease. Specifically, hydroxyproline assays revealed that Methylnissolin at concentrations of 12.5, 25, and 50 μM inhibited the secretion of type IV collagen. Type IV collagen is an important component of the glomerular basement membrane. In a high glucose environment, the proliferation of glomerular mesangial cells and the excessive synthesis of type IV collagen can lead to thickening of the basement membrane and glomerular fibrosis, thereby exacerbating the pathological progression of diabetic kidney disease.[53] However, the specific mechanism by which Methylnissolin inhibits type IV collagen remains unclear, although it is known to be possibly related to its hypoglycemic activity. This is because, in α-glucosidase inhibition assays, Methylnissolin, rather than Methylnissolin-3-O-glucoside, exhibits significant inhibitory activity at a concentration of 100 μM.[54] This effect may indirectly mitigate high glucose-driven oxidative stress and advanced glycation end-product formation, both of which exacerbate mesangial cell dysfunction and collagen overproduction. Furthermore, Methylnissolin at 100 μM also inhibits lipid accumulation, with effects comparable to the positive control (6-gingerol, 25 μM).[41] Western blot analysis revealed downregulation of adipogenic transcription factors C/EBPα, C/EBPβ, and PPARγ—master regulators of adipocyte differentiation and lipid storage. By suppressing this transcriptional triad, Methylnissolin disrupts lipid synthesis pathways, potentially ameliorating lipotoxicity-induced renal injury. These findings

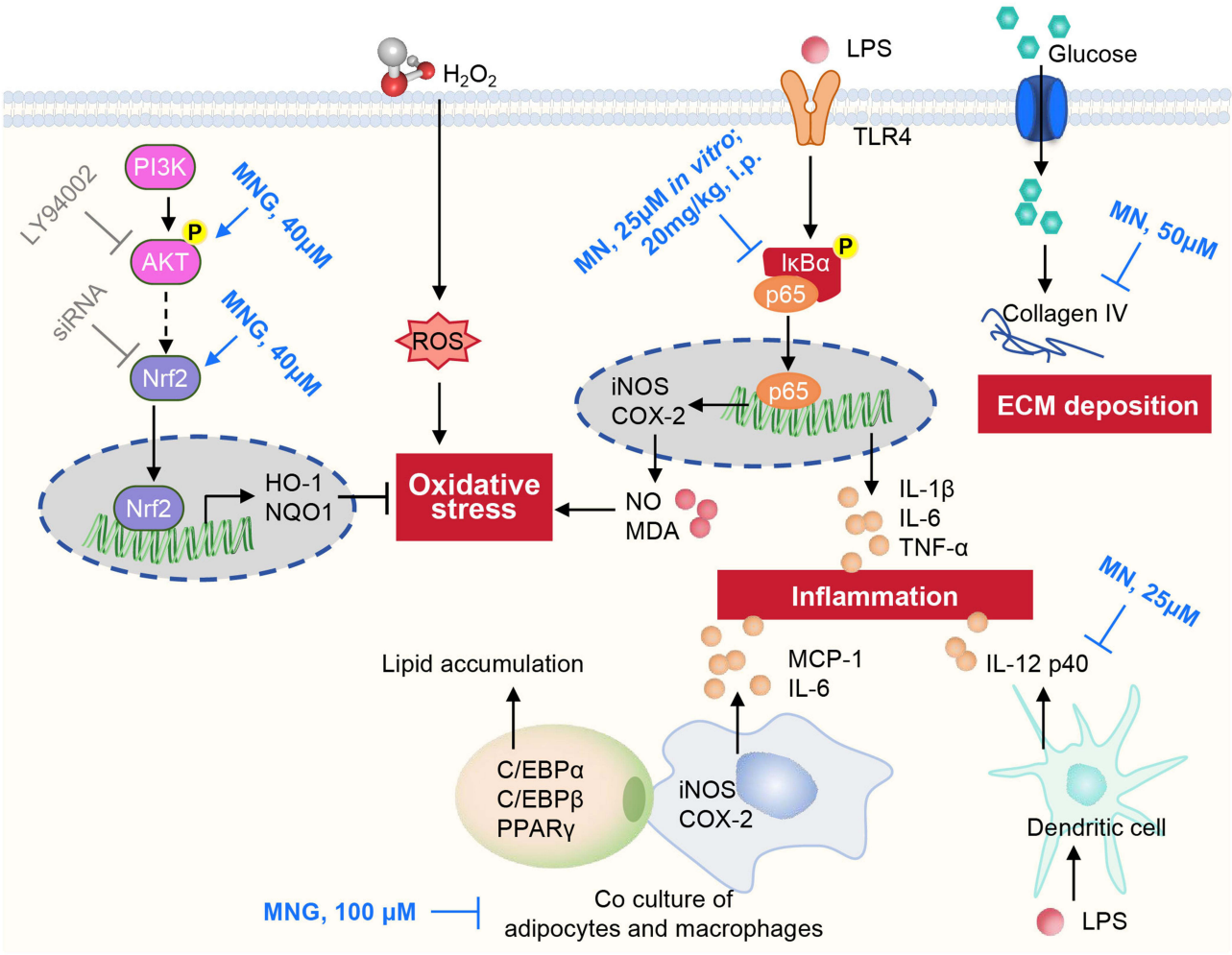

Figure 2 Potential mechanisms underlying the antioxidant, anti-inflammatory, and other activities of Methylnissolin and its glycosides. Established functions are denoted by solid arrows, whereas indirect effects are indicated by dashed lines. Gray italic text represents interventions in the pathway, such as inhibitors, gene overexpression, or silencing. Adapted from Smart Servier Medical Art. https://smart.servier.com/. https://creativecommons.org/licenses/by/4.0/.[51]
Abbreviations: MN, Methylnissolin; MGN, Methylnissolin-3-O-glucoside; i.p., intraperitoneally; ECM, extracellular matrix deposition.

underscore the potential application of Methylnissolin in improving disorders of glucose and lipid metabolism. Further studies are needed to validate whether these effects involve upstream signaling nodes such as AMPK activation.

Antitumor

Methylnissolin demonstrates multifaceted antitumor activity through distinct molecular mechanisms. Through the CCK8 cytotoxicity assay, it was found that Methylnissolin exhibits a time- and dose-dependent inhibitory effect on cervical cancer SiHa cells, with a 48-hour IC50 value of 187.40 μM.[55] Flow cytometry analysis further showed that Methylnissolin predominantly induces early apoptosis (81.01% vs control 14.91%), with minimal late apoptotic effects (7.06% vs control 4.56%), suggesting activation of intrinsic mitochondrial apoptosis pathways rather than external death receptor signaling. The role of p53 in cell cycle regulation is mainly reflected in the G1/S and G2/M checkpoints. Although Methylnissolin significantly reduced the proportion of cells in the S phase, causing more cells to be arrested in the G2/M phase, it had no impact on the expression level of p53.[55] It is preliminarily speculated that the regulation of cell proliferation by Methylnissolin may be achieved through mechanisms other than p53, such as cyclin-dependent kinases (CDKs). High-throughput virtual screening identified CDK2, a key driver of G1/S transition, as the top-ranked binding partner for Methylnissolin, with a binding free energy of −8.5 kcal/mol.[55] Molecular docking revealed a stable hydrogen bond between Methylnissolin's hydroxyl group and ASP-145 in CDK2's catalytic pocket—a residue critical for ATP binding. By

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occupying this site, Methylnissolin likely disrupts CDK2-cyclin E complex formation, thereby blocking S-phase entry. This aligns with the observed S-phase depletion and G2/M accumulation. Besides cervical cancer, a network pharmacology analysis by Feng et al revealed that Methylnissolin enriched numerous targets in metastatic colon cancer, with a degree value of 25.[56] However, there is a lack of sufficient in vivo and in vitro experimental validation results. Currently, only two in vivo studies have revealed the anticancer effects of Methylnissolin and its glycoside. At a dose of 40 mg/kg, Methylnissolin alleviated cachectic symptoms in a mouse gastric cancer model, including reduced weight loss and loss of muscle and white adipose tissue, as well as damage to liver and kidney functions. The mechanism of action may be achieved by targeting the RIPK2/ASK1 pathway.[57] Recently, in a patient-derived xenograft mouse model of esophageal cancer, intraperitoneal injection of 10mg/kg Methylnissolin-3-O-glucoside for 18 days effectively inhibited tumor progression.[58] A dose as high as 60 mg/kg did not cause significant weight loss or organ damage in tumor-bearing mice. Further molecular docking, pull-down assays, Drug affinity responsive target stabilization assays, Cellular thermal shift assay, and RNA immunoprecipitation revealed that Methylnissolin-3-O-glucoside can competitively bind to DDX5 at the K144 site (see Figure 3). Therefore, the mechanism of action of Methylnissolin-3-O-glucoside may involve inhibiting the binding of DDX5 with VAV3, thereby suppressing the progression of esophageal squamous cell carcinoma.

Enzyme Inhibition Activity

Cell signaling, metabolic regulation, cell growth, differentiation, and apoptosis processes all depend on the action of various enzymes. Understanding how Methylnissolin affects the activity of these enzymes is crucial for drug development. Liu et al discovered that Methylnissolin may have dual-target inhibitory effects on the interaction between active ingredients and enzymes.[59] Molecular docking simulations in this study showed binding energies of −8.5 and −5.3 kcal/mol for Acetylcholinesterase and 5-Lipoxygenase, respectively. A network pharmacology analysis targeting vitiligo

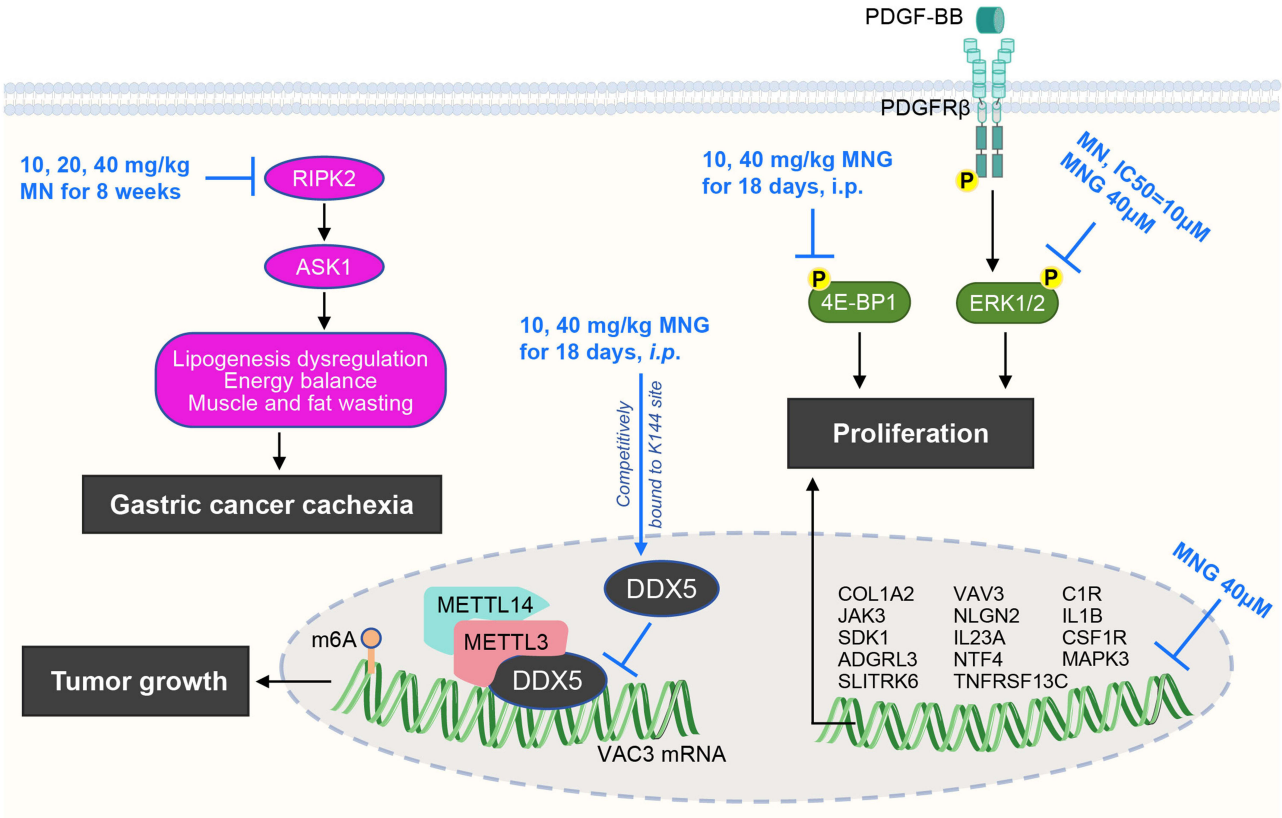

Figure 3 Potential mechanisms of Methylnissolin and its glycosides in tumor suppression. Adapted from Smart Servier Medical Art. https://smart.servier.com/. https://creativecommons.org/licenses/by/4.0/.[51]

Abbreviations: MN, Methylnissolin; MGN, Methylnissolin-3-O-glucoside; i.p., intraperitoneally. 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; RIPK2, receptor interacting protein kinase 2; ASK1, apoptosis signal-regulating kinase 1.

revealed that Methylnissolin-3-O-glucoside might be the active component regulating key kinases, including phosphatidylinositol 3-kinase, protein kinase B, mitogen-activated protein kinases, and mTOR kinase.[60] These bioinformatics results were validated by another in vitro study using A10 cells. Methylnissolin at 10 μM was able to inhibit PDGF-BB-induced phosphorylation of ERK1/2, thereby suppressing cell proliferation and DNA synthesis.[61] Notably, in this in vitro model, Methylnissolin's effects did not influence the phosphorylation of PDGF-β-receptor, Akt kinase, and p38 MAP kinase, initially indicating the selectivity of Methylnissolin's action. The SwissADME prediction tool, based on computer algorithms, showed that Methylnissolin, but not methylnissolin-3-O-glucoside, has potential inhibitory activity on multiple CYP enzymes, including CYP1A2, CYP2C19, CYP2D6, and CYP3A4. Preliminary suggest that Methylnissolin may pose a higher risk in terms of drug interactions.

Pharmacokinetics

Absorption

Xu et al were the first to report the absorption of Methylnissolin and its glycoside using in vitro experiments.[62] Methylnissolin was detected in both the modified rat everted sac experiment and the Caco-2 cell monolayer model experiment. Methylnissolin-3-O-glucoside was only detected in the modified rat everted sac experiment. Subsequent studies have reported their absorption in various Chinese herbal decoctions containing *Astragalus mongholicus*. For example, Methylnissolin-3-O-glucoside and Methylnissolin were simultaneously detected in the serum of rats orally administered Fangji Huangqi Decoction (1.5 g/100 g, twice daily for 3 days) using UPLC-HRMS/MS.[63] Rabbits were orally administered Danggui Huoxue Decoction (*Astragalus mongholicus: Angelica sinensis (Oliv). Diels* = 5:1) at a concentration of 1.8 g/mL with a dose of 50 g/kg. The blood analysis using metabolite fingerprinting technology and liquid chromatography/diode array detection mass spectrometry revealed that Methylnissolin-3-O-glucoside is an absorbed component.[64] In situ liver cancer rats orally administered Fuzheng Yiliu Decoction (containing 150g *Astragalus mongholicus*) for 2 weeks had exogenous metabolites methylnissolin detected in their blood.[65] These results are consistent with the predictions from SwissADME, suggesting that computational chemistry prediction methods can be used for pre-screening of orally active compounds, saving time and money. However, these methods cannot predict compounds that are absorbed via active transport and do not consider compound concentration.

Currently, only two pharmacokinetic studies involving Methylnissolin and its glycosides have been reported. After rats were administered Bufei Huoxue capsules (with *Astragalus mongholicus* as the principal herb), blood samples were collected at 15 different time points for UHPLC-MS/MS analysis.[66] The $T_{max/h}$, $T_{1/2z/h}$, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and $MRT_{0-t/h}$ of Methylnissolin were 0.74 ± 0.59 h, 4.88 ± 1.82 h, 1.04 ± 0.26 μg/L, 5.69 ± 1.41 μg/L·h, 6.10 ± 1.52 μg/L·h, and 5.97 ± 1.71, respectively. This suggests that Methylnissolin has characteristics of fast absorption and prolonged retention time in the body. The concentration-time curve of Methylnissolin shows a "double peak" at 30 minutes and 4 hours. The double-peak phenomenon is not uncommon in traditional Chinese medicine (TCM) compound prescriptions. Due to the interactions and synergistic effects of multiple components, the kinetic behavior of TCM compound drugs often exhibits different characteristics compared to individual components. The result was confirmed by another study. Compared to the *Astragalus mongholicus* alone, the combination (*Astragalus mongholicus* - *Saposhnikoviae Radix* pair group) intensified the double-peak phenomena of Methylnissolin.[67] For Methylnissolin-3-O-glucoside, the extent of in vivo exposure, residence time, and half-life in the combination group were significantly increased.[67] However, there is still a lack of systematic pharmacokinetic studies on the oral administration of Methylnissolin alone. This gap limits our deeper understanding of its pharmacological mechanisms and provides insufficient basis for drug development. Further studying the pharmacokinetic characteristics of Methylnissolin will help understand its effects in vivo, thus providing a scientific basis for optimizing dosage and administration regimens.

Metabolism and Excretion

In a modified rat everted sac experiment, it was found that Methylnissolin can be absorbed and metabolized by the intestine, with glucuronic acid compounds as its main metabolites.[62] This result was further confirmed in another in vivo

experiment. Using UPLC-Q-TOF/MS technology, glucuronic acid products of Methylnissolin were detected in rat plasma.[60] Additionally, a rat liver S9 incubation system was used to simulate the Phase I metabolism of Methylnissolin. A total of 40 metabolites and 1 degradation product (named 4-methoxy-astrai-soflavan) were identified.[68] The major metabolites of Methylnissolin, ranked by relative content, are 6-hydroxy-Methylnissolin, diastereomer of 6-hydroxy-Methylnissolin, astrametabolin I, 9-demethyl-Methylnissolin, 1/2/4/6/11 monohydroxylated demethylated Methylnissolin, and Methylnissolin dimer. The percentages of the total peak area are 19.62%, 14.10%, 14.01%, 11.49%, 8.35%, and 4.96%, respectively. The main metabolic reactions include hydroxylation, demethylation, dimerization, hydration, and dehydrogenation, as shown in Figure 4. Notably, Methylnissolin is also excreted in its prototype form. After oral administration of Buyang Huanwu Decoction (containing 16kg of *Astragalus mongholicus*) to Wuzhishan miniature pigs, 11mg of Methylinsson was detected in 7g of 20% methanol urine extract.[17] In a 5g 70% methanol urine extract, 11mg of glucuronic acid compounds of Methylinsson were detected. This glucuronidated metabolite has also been detected in various biological samples, including human urine,[62] pig urine and serum,[17,69] rat serum,[63] plasma,[60] everted sac experiments,[62] urine, and bile.[16] Studies combining fecal metabolomics and systems pharmacology have shown that Methylnissolin in *Astragalus mongholicus* mainly affects the biosynthesis/degradation of valine, leucine, and isoleucine,[70] possibly by acting on multiple metabolic enzymes, including l-serine dehydratase, branched-chain amino acid aminotransferase, 3-hydroxyacyl-CoA dehydrogenase, isovaleryl-CoA dehydrogenase, and acetyl-CoA C-acetyltransferase. Therefore, the above reports may indicate that Methylinsson is an original isoflavone present in *Astragalus mongholicus* and an important absorbed component, excreted component, and metabolic precursor in the body.

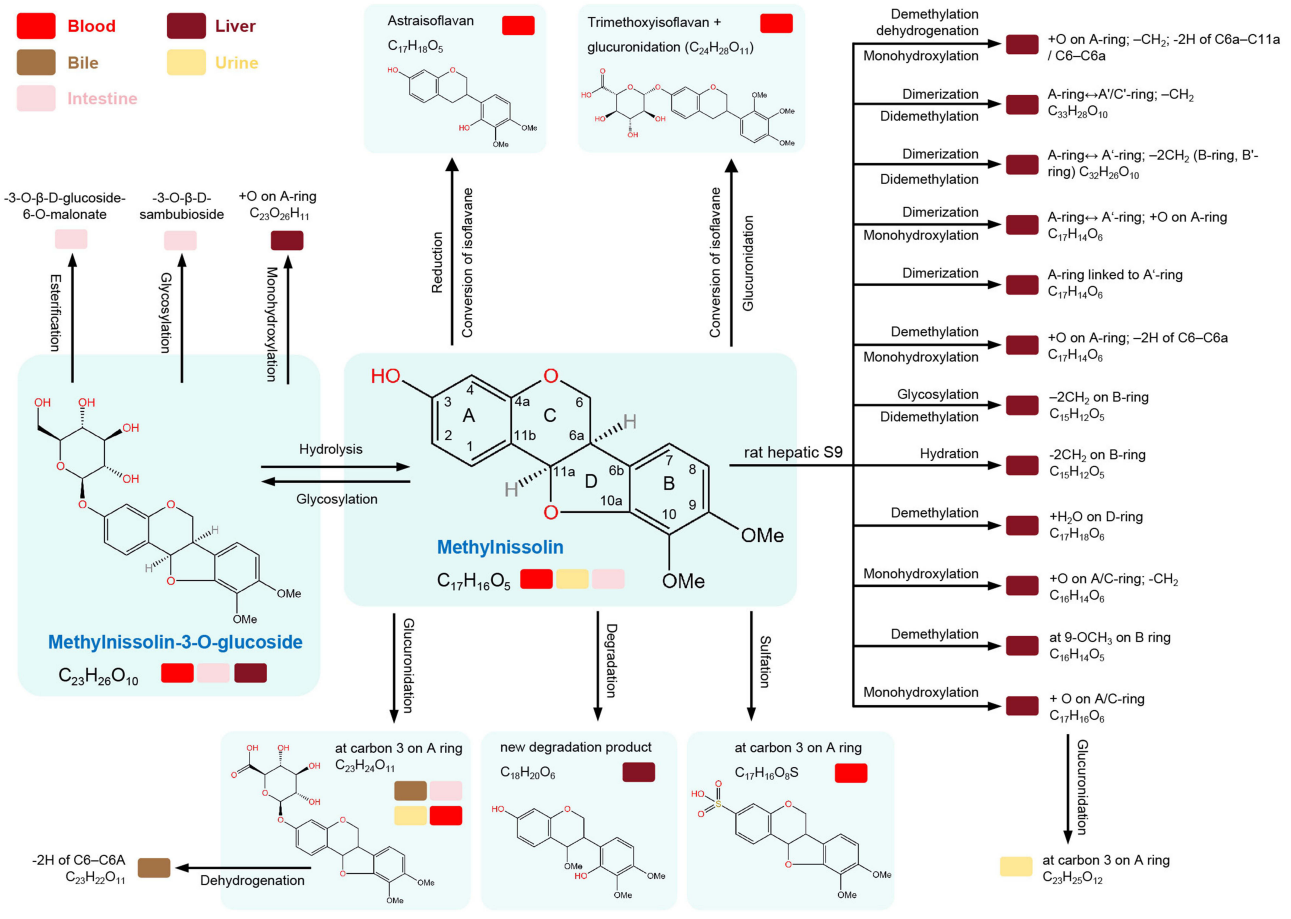

Figure 4 The metabolic reaction and products of methylnissolin.

Toxicological Profile and Safety Assessment

Current toxicological data on methylnissolin and its glycosides remain limited. In vitro studies have utilized concentrations of 3.3–100 μM for mechanistic analyses,[41,42,46,52] reporting no significant cytotoxicity within this range. The half-maximal inhibitory concentration (IC_{50}), an indicator of compound potency to inhibit 50% of a biological process, may indirectly reflect cytotoxic potential. For instance, methylnissolin exhibited an IC50 of 187.4 μM against SiHa cervical cancer cells after 48 hours.[55] Similarly, methylnissolin-3-O-glucoside demonstrated IC50 values of 186.44 μM (24 h) and 119.23 μM (48 h) in KYSE150 esophageal carcinoma cells, with lower toxicity in normal esophageal epithelial cells (IC50 = 293.27 μM at 24 h; 172.91 μM at 48 h).

ADMETlab3 and ProTox-3.0, two widely adopted computational toxicity prediction platforms, leverage machine learning and structural descriptors to predict compound toxicity by benchmarking against known toxicants. ADMETlab3 flagged methylnissolin-3-O-glucoside as high-risk for Ames mutagenicity (probability: 0.915) and skin sensitization (probability: 0.988).[71] In contrast, ProTox-3.0 classified methylnissolin-3-O-glucoside as low-risk for acute oral toxicity (LD_{50} = 3000 mg/kg; Toxicity Class 5) and hepatotoxicity.[71] Methylnissolin exhibited moderate risks for drug-induced liver injury (probability: 0.623) and Ames mutagenicity (probability: 0.583), alongside active immunotoxicity and respiratory toxicity.

In murine models, Shi et al evaluated methylnissolin-3-O-glucoside via intraperitoneal injection at doses of 5–60 mg/kg for 7 days.[58] While a gradual weight loss trend was observed at 60 mg/kg, no statistically significant differences were detected compared to the control group (0 mg/kg). Histopathological examination (H&E staining) revealed no organ-specific lesions in the heart, liver, spleen, lungs, or kidneys. These findings support short-term tolerability within tested doses; however, potential risks associated with higher doses or chronic exposure require further investigation.

Factors Influencing Methylnissolin Content

Variety and Origin

The current research focuses on comparing two common varieties of *Astragalus mongholicus* root: *Astragalus membranaceus var. mongholicus (Bge). Hsiao* (AMM) and *Astragalus membranaceus (Fisch). Bge*. (AM). High-performance liquid chromatography-electrospray method found that AMM and AM contain almost the same total flavonoid compounds.[72] However, the methylnissolin-3-O-glucoside and methylnissolin in AM are higher than those in AMM. The comparison of producing areas mainly consists of four notable regions in China: Heilongjiang, Inner Mongolia, Shanxi, and Gansu. Researchers detected the cultivated AM varieties using LC-MS/MS and found that the region with the highest total isoflavone content was Heilongjiang, followed by Inner Mongolia.[73] To compare the differences in methylnissolin and its glycoside, we extracted the data from this study for further analysis. We calculated the relative percentage by dividing the content of methylnissolin or methylnissolin-3-O-glucoside measured in the study by the total isoflavone. The results showed that the methylnissolin-3-O-glucoside was highest in Shanxi (as shown in Figure 5A).

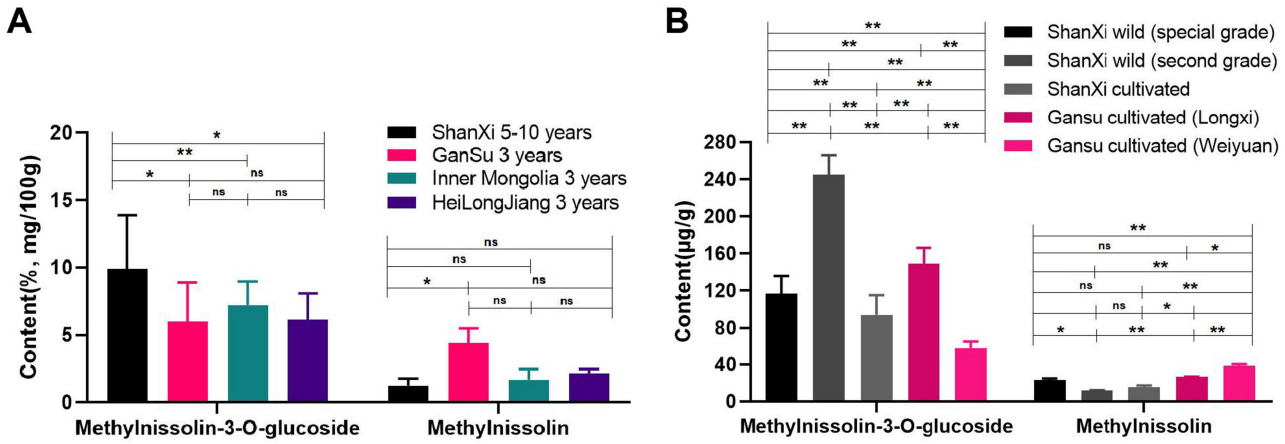

Figure 5 Differences in the content of methylnissolin and its glycosides in *Astragalus mongholicus* from different origins. (**A**), data extracted from the study by Song et al[73] (**B**), data extracted from the study by Cui et al.[74] Data are shown as the mean ± sd. Statistical analyses were performed using two-way ANOVA. * P < 0.05, ** P < 0.01.

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This might be due to the cultivation age, as the samples collected from Shanxi were 5–10 years old, while those from other regions were 3 years old. However, the methylnissolin content in Shanxi was the lowest and significantly lower than that in Gansu ($p<0.05$). Interestingly, the content of these two compounds was completely opposite. The content order of methylnissolin-3-O-glucoside was Shanxi > Inner Mongolia > Heilongjiang > Gansu. The content order of methylnissolin was Gansu > Heilongjiang > Inner Mongolia > Shanxi. This conclusion was supported by another research team,[74] as shown in Figure 5B. The study compared *Astragalus mongholicus* from five sources and found that Methylnissolin-3-O-glucoside was highest in the wild species from Hunyuan, Shanxi, and lowest in the cultivated species from Weiyuan, Gansu. Conversely, Methylnissolin was highest in the cultivated species from Weiyuan, Gansu, and lowest in the wild species from Hunyuan, Shanxi.[74] Additionally, Zhao et al conducted a quality differentiation analysis of AMM from four notable regions.[75] The results showed significant differences in methylnissolin-3-O-glucoside between wild and cultivated AMM. Based on cluster analysis and principal component analysis, methylnissolin-3-O-glucoside clustered towards the cultivated species. The authors believe this is one of the significant variables contributing to distinguishing between wild and cultivated *Astragalus mongholicus*. It may assist in assessing the quality of both wild and cultivated *Astragalus mongholicus*, as well as evaluating how closely cultivated specimens resemble their wild counterparts.

Extraction Site

The total isoflavone content showed no significant difference between taproot and branch root, or between the xylem and bark of roots in four recognized production regions.[73] Further studies on different parts of the root revealed that the xylem or bark did not have a significant impact on the Methylnissolin content across different regions (Figures 6A–D). A significant observation was made in the Shanxi region where the methylnissolin-3-O-glucoside content in the xylem was notably higher than in the bark (Figure 6A).[76] Another study compared different extraction parts of AMM and AM from the same origin (Gansu, China). For Methylnissolin-3-O-glucoside levels, the Stem and Leaf parts of AMM had higher content than AM (Figure 6E). Methylnissolin was mainly concentrated in the roots, with the remaining parts (Rhizome, Stem, Leaf, and flower) having higher content in AMM than in AM (Figure 6F). The research results may help in the rational utilization of bioactive components in AMM and AM resources. Although the root is the main medicinal part of *Astragalus mongholicus*, the stem, leaf, or flower could be more economical materials for extracting Methylnissolin and its glycosides.

Processing Method

A recent study evaluated the impact of chlormequat treatment on 13 major active ingredients of *Astragalus membranaceus* collected in Gansu, China.[77] It was found that different concentrations of chlormequat had varying effects on the active ingredients of *Astragalus membranaceus*, with the content of methylnissolin-3-O-glucoside showing a significant decrease (22.18 ~ 41.69%). Li et al reported the impact brought by extraction solvents. Under the same extraction method, the content of methylnissolin-3-O-glucoside obtained using ethanol as the solvent was about 1.5 times higher than that obtained using water.[45] In addition, a non-targeted rapid resolution liquid chromatography coupled with quadrupole time-of-flight mass spectrometry method based on metabolomics was used to study the chemical changes in *Astragalus membranaceus* (originating from Mongolia and Shanxi) resulting from roasting process.[78] The results showed that various derivatives of Methylnissolin were obtained after roasting, with significant increases in the contents of Methylnissolin-3-O-glucoside, Methylnissolin-3-O-GIc-6'-O-Ac-2'-O-xyI, and Methylnissolin-3-O-GIc-6"-O-Ac, while Methylnissolin-3-O-Glc-6-O-Mal and Methylnissolin-3-O-Glc-6'-O-Mal-2'-O-xyI significantly decreased. The possible chemical transformation mechanism is that the malonyl isoflavones of pterocarpus can be converted into the corresponding acetyl isoflavones (mainly) and glycosides (secondarily).[78]

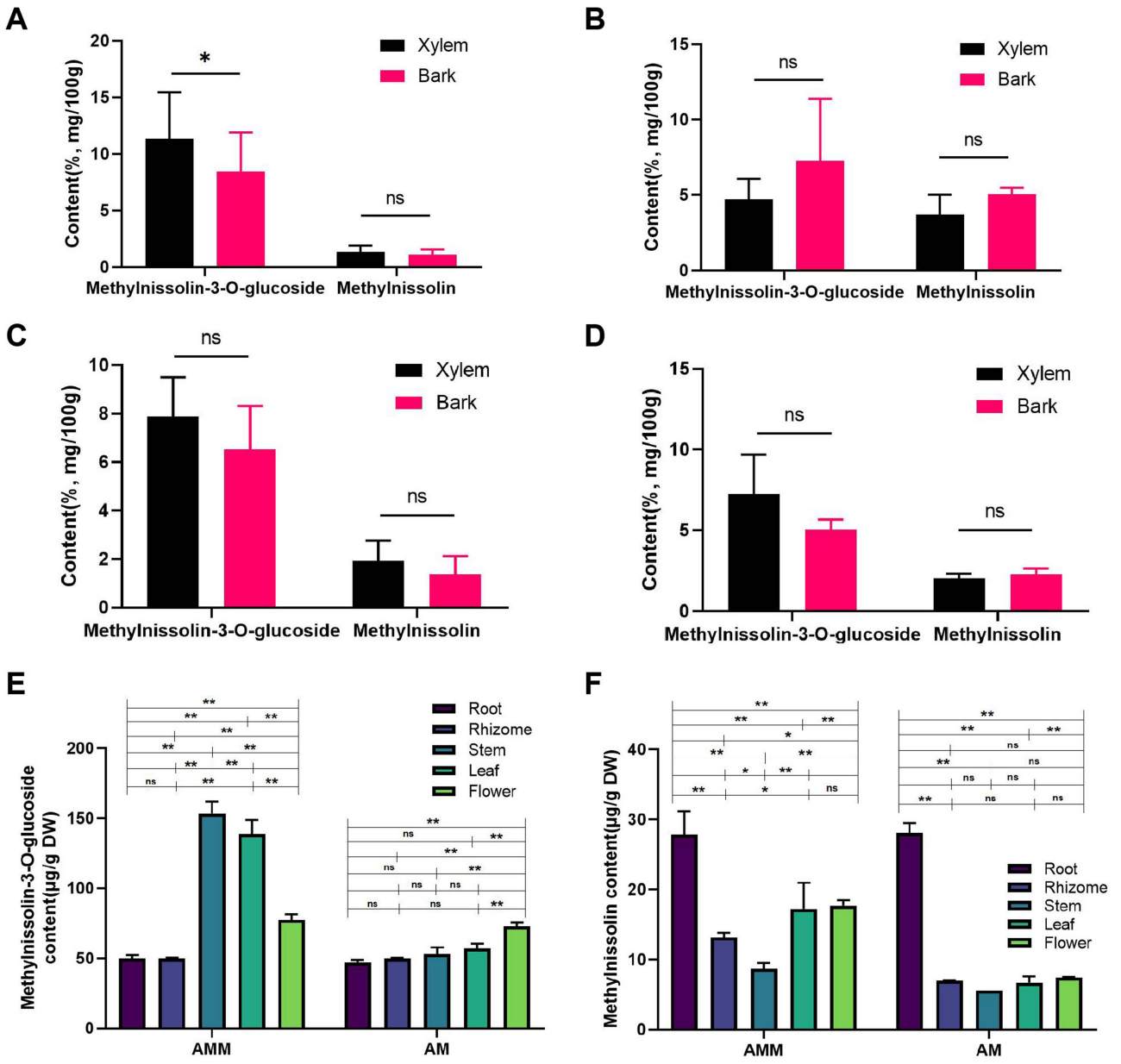

Figure 6 Differences in the content of Methylnissolin and its glycosides in different extract parts of *Astragalus membranaceus*. The content of methylnissolin and methylnissolin-3-O-glucoside in the xylem and bark of *Astragalus membranaceus* from Shanxi (**A**), Gansu (**B**), Inner Mongolia (**C**), and Heilongjiang (**D**) regions of China. Data extracted from the study by Song et al.[73] The content of (**E**) Methylnissolin-3-O-glucoside and (**F**) Methylnissolin in roots, rhizome, stem, leaf, and flower. Data extracted from the study by Li et al.[76] Data are shown as the mean ± sd. Statistical analyses were performed using two-way ANOVA. * $P < 0.05$, ** $P < 0.01$.

Conclusion and Perspective

Methylnissolin and Methylnissolin-3-O-glucoside derived from the leguminous plant Astragalus have great potential for drug development. Both are important blood-entry components of *Astragalus membranaceus* and exert effects through signaling pathways such as RIPK2/ASK1, PI3K/AKT, IκB/NF-κB, MAPK, and NRF2/HO-1. They encompass pharmacological activities including anti-inflammatory, antioxidant, regulation of glucose and lipid metabolism, and antitumor effects, showing promising applications in disease treatment. Compared to Methylnissolin, Methylnissolin-3-O-glucoside focuses more on exhibiting antioxidant bioactivity. Methylnissolin, on the other hand, has higher lipophilicity, can be absorbed by the intestines, and is excreted in its prototype form through urine. Methylnissolin is an important metabolic precursor, involved in metabolic reactions in the body including glycosylation, monohydroxylation, glucuronidation, didemethylation, sulfation, and dimerization.

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However, systematic pharmacokinetic studies on the oral administration of Methylnissolin and Methylnissolin-3-O-glucoside are lacking. The computer prediction that Methylnissolin acts as an inhibitor of several cytochrome P450 enzymes, specifically CYP1A2, CYP2C19, CYP2D6, and CYP3A4, highlights the importance of investigating potential drug-drug interactions. Understanding how Methylnissolin influences the metabolism of co-administered medications will be crucial for its safe therapeutic application. Methylnissolin and its glycosides have demonstrated highly attractive intervention effects on diabetes, cancer, and immune inflammatory diseases. Future research should focus on elucidating the detailed molecular mechanisms through which Methylnissolin interacts with its targets. Exploring the synergistic effects of Methylnissolin with other compounds, both within Astragalus and from other sources, could uncover novel therapeutic combinations. Given the preliminary toxicological data, long-term toxicity studies and genotoxicity assays are essential to define safe dosage windows and assess potential impacts on hepatic, renal, and immune systems. In addition, the establishment of standardized extraction methods and resource utilization studies will aid in the rational development of *Astragalus membranaceus* resources. Stems, leaves, or flowers may be more economical materials for extracting Methylnissolin and its glycosides. These efforts will deepen our understanding of Methylnissolin's therapeutic potential and pave the way for its clinical application.

Abbreviations

AMM, *Astragalus membranaceus var. mongholicus (Bge.) Hsiao*; AM, *Astragalus membranaceus (Fisch.) Bge*; ASK1, apoptosis signal-regulating kinase 1; CDK, cyclin-dependent kinases; ECM, extracellular matrix deposition; IL, interleukin; i.p., intraperitoneally; IC50, half maximal inhibitory concentration; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; MN, Methylnissolin; MGN, Methylnissolin-3-O-glucoside; RIPK2, receptor interacting protein kinase 2; TCM, traditional Chinese medicine; TPSA, total polar surface area; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1.

Consent for Publication

All participants agree to publish.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that there are no conflicts of interest.

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