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Review article

A review of *Lycium ruthenicum* Murray: Geographic distribution tracing, bioactive components, and functional properties

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ABSTRACT

Lycium ruthenicum (LRM), endemic to Northwest China, is known as hei goji or black goji and is renowned for its rich bioactive compounds. This review analyzes LRM's geographic distribution and traceability and highlights challenges and future developments in geographical traceability. The work also focuses on LRM's bioactive constituents, especially on anthocyanins and polysaccharides, demonstrating a clear clue for understanding their updated extraction methods, identification, and diverse bioactive activities, including antioxidation, anti-inflammation, and immunomodulation, which is beneficial to developing novel functional foods and new medical materials. Moreover, the paper elucidates advances in the potential application of LRM in food preservation, packaging, and other domains. Notably, we figure out gaps in LRM research, such as traceability technology and the proven efficacy of biological activities. This study provides a foundation for future perspectives on developing nutraceuticals and functional foods, disease treatment supplements, and green food packaging materials by bridging these gaps.

1. Introduction

The growing interest in plant-based functional foods is driven by their abundant content of phytochemicals and bioactive components, such as polyphenols, polysaccharides, and sterols, which play a significant role in health promotion and nutritional supplement [1]. Among these, LRM, enriched with anthocyanins and polysaccharides, emerges as a significant role in the realm of health promotion and nutritional supplement. Recognized for its diverse bioactive compounds [2,3], LRM serves not only as a staple in traditional Chinese medicine but also stands at the forefront of functional food research.

Lycium, belonging to the Solanaceae family, includes 97 species, among which LRM, also known as black wolfberry, hei goji, or black goji, is used as a traditional herb in China [4]. Given the potential health-promoting effects associated with LRM, there has been a growing interest among consumers and businesses. However, the prices of LRM vary significantly among different geographical origins, with those from Qinghai province typically commanding the highest prices. Consumers prefer low-yield wild LRM, leading to the illegal sale of counterfeit high-quality LRM, which threatens consumer interests. Researchers have thus investigated distinguishing between wild strains and cultivated variants using various identification techniques, though practical commercial application remains challenging and requires further development.

Research on the bioactive compounds of LRM has primarily focused on anthocyanins and polysaccharides, particularly their

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extraction [5], identification [5,6], biological activities [7–9], and health-promoting effects [8,10–14]. This includes investigating environmentally friendly and cost-effective extraction methods to enhance efficiency and minimize damage to the extracts [5,6,15,16]. Early studies explored their general biological activities such as antioxidant and antibacterial anti-inflammatory effects [17–19], while recent research has examined their potential therapeutic effects on specific diseases in vitro and in vivo [12,20], including mice model trials [13,14,21]·

In terms of functional applications, research has concentrated on the potential use of LRM extracts in food preservation and active packaging. This includes exploring the efficacy of LRM extract active films in indicating the freshness of common foods such as shrimp [22], milk [23], and chicken [24], as well as the role of LRM extracts in delaying food spoilage or inhibiting microbial [25,26]. Enhancing the stability of such novel packaging to ensure its industrial applicability remains a key direction for future research.

To our knowledge, several review articles on LRM have been published. Yao et al. and Wang et al. provided foundational insights into its botanical and biochemical facets [2,4], while more recent studies have begun to explore innovative applications, such as using monomeric anthocyanin petanin as a natural colorant [3]. Despite the rich body of literature on LRM, comprehensive studies that integrate the traceability of its geographical origins with an analysis of its bioactive compounds and potential applications of LRM extracts are lacking. This gap marks a critical area of research that could bridge traditional uses and modern scientific validation, providing a clear trajectory for future investigations aimed at harnessing LRM's full potential. Thus, this study seeks to integrate these diverse strands of research, offering novel insights into the optimization of LRM's extraction processes, enhancing its bioavailability, and expanding its applications in nutraceuticals and functional foods. By doing these, it aims to not only further our understanding of LRM but also to leverage its economic, nutritional, and medicinal properties to meet growing demands.

2. Methodology

2.1. Literature search and selection criteria

To compile a comprehensive review of LRM, we conducted a systematic literature search using multiple academic databases including PubMed, Scopus, and Web of Science. The search was performed using the following keywords: "*Lycium ruthenicum* Murray," "black wolfberry," "bioactive compounds," "anthocyanins," "polysaccharides, "extraction", "characterization", "biological activities","geographical distribution"," "food preservation," "food preservation," and "functional foods." The search was limited to articles published in English.

2.1.1. Inclusion criteria

- Peer-reviewed articles
- Research concentrating on Traceability of geographical distribution of LRM
- Studies focusing on the extraction, characterization, and function of LRM components
- Research on the applications of LRM in functional food, food preservation and health and other relative domains.

2.1.2. Exclusion criteria

- Articles not available in full text
- Studies not directly related to LRM or its bioactive compounds
- Non-peer-reviewed sources

2.2. Article selection process

Our initial search yielded 506 articles. After removing duplicates and screening titles and abstracts for relevance, 200 articles were shortlisted for full-text review. Following a detailed evaluation based on our inclusion and exclusion criteria, 108 articles were included in this review. The references were updated to include the most recent studies available up to 2024.

3. Traceability of geographical distribution

LRM is distributed across a diverse range of climates and geographies, extending from Northwest China to various regions such as Iran, Afghanistan, India, Mexico, Pakistan, Russia, Turkmenistan, and Georgia [4]. Predominantly native to China, LRM is believed to have originated from America before dispersing across Eurasia, adapting to environments characterized by significant drought and salt stress [2]. It thrives particularly in China's arid Northwest, notably in Qinghai and Ningxia provinces, where it serves both as a valuable nutritional source and a critical component in ecological management strategies such as soil erosion control. Qinghai Province has become renowned for producing high-quality LRM, attributed to its ideal climatic and geographical conditions. The LRM from this region not only boasts superior nutritional properties but also commands higher market prices compared to those sourced from other regions, reflecting its premium quality [17]. However, the market for LRM is currently compromised by issues of mislabeling and adulteration, primarily driven by economic incentives. These malpractices damage the reputation of high-quality LRM, diminish consumer trust, and raise serious food safety concerns [27].

To combat these challenges, robust traceability methods are essential. Chromatographic techniques have proven effective in tracing the geographical origins of LRM, with studies showing that variations in anthocyanin profiles can serve as reliable indicators of geographic origin. These variations are influenced by genetic, geographic, and climatic factors, thus providing a scientific basis for authentication [17,28]. Near-Infrared Spectroscopy (NIRS) and related chemometric methods such as Synergy Interval Partial Least Squares (Si-PLS) and Least-Squares Support Vector Machine (LS-SVM) are being explored for their potential to non-destructively predict anthocyanin content and verify geographical origins, marking a promising direction for future applications [29]. Furthermore, the application of electronic nose (e-nose) and electronic tongue (e-tongue) technologies, along with advanced multivariate statistical analyses like PCA and Linear Discriminant Analysis (LDA), offers rapid and cost-effective alternatives for geographical origin verification. These techniques reduce the need for extensive sample preparation and reliance on expensive instrumentation [30]. Recent advancements include the use of High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) systems and Ultra Performance Liquid Chromatography-triple Quadrupole-Time Of Flight-Mass Spectrometry (UPLC-triple Q-TOF-MS) combined with Principal Component Analysis (PCA). These methods have refined the discrimination models, enhancing the ability to distinguish LRM samples from different provinces based on their specific anthocyanin concentrations, with notable distinctions identified in samples from Qinghai [31]. Innovative approaches like vis-NIR spectroscopy, particularly when combined with chemometric techniques such as Partial Least Squares Regression (PLSR), have also shown potential in detecting adulteration levels in related products like desiccated coconut powder, suggesting possible applications for LRM traceability [32].

4. Bioactive components

LRM is renowned for its rich array of bioactive components, which include anthocyanins, phenolic acids, polysaccharides, carotenoids, fatty acids, phytosterols, and alkaloids. These compounds have been extensively studied for their extraction methods, identification processes, and biological activities, all aimed at harnessing their nutraceutical and health-promoting potentials. The most emphasized compounds are anthocyanins and polysaccharides. The LRM bioactive compounds and their bioactive activities are summarized in Table 1.

4.1. Phenolic compounds

Phenolic compounds are vital secondary metabolites in plants, celebrated for their significant contributions to human health. LRM is particularly abundant in these compounds, including a variety of flavonoids and phenolic acids, which play pivotal roles in antioxidation, anti-inflammation, and immunomodulation. Remarkably, LRM exhibits a higher phenolic content and antioxidant capacity compared to Lycium barbarum, underscoring its superior efficacy as a source of natural antioxidants [33].The adoption of ultrasound-assisted extraction (UAE) represents a significant improvement over traditional solvent extraction methods, standing out for its environmental friendliness, simplicity, cost-effectiveness, and enhanced efficiency in extracting higher yields of phenolic compounds [34]. In the domain of phenolic compound identification, techniques such as UPLC-Q-Orbitrap MS and HPLC fingerprinting combined with Quadrupole-Time of Flight-Electrospray Ionization-Mass Spectrometry (Q-TOF-ESI-MS) are highly valued for their precision and sensitivity [28,35].

The efficacy of phenolic compounds in exerting antioxidant activities has been confirmed [34,35]. It also have demonstrated the protective capabilities of these phenolics against oxidative stress in human intestinal tissue-derived Caco-2 cells and PC12 cells [36].

Although the collective biological activities of LRM's total phenolics are still under study, the current scientific focus tends to gravitate towards individual phenolic compounds, especially anthocyanins. This narrower focus facilitates a deeper understanding of the specific bioactivities associated with these compounds and promotes the development of novel functional foods that can effectively utilize these bioactivities to confer health benefits.

4.1.1. Anthocyanins

Anthocyanins, a subgroup of flavonoids found in LRM [37], are pivotal active compounds garnering substantial attention for their extraction, characterization, and health-promoting impacts. The extraction, purification, and identification for LRM anthocyanins are summarized in Table 2.

4.1.1.1. Extraction methods. The chemical structure of anthocyanins is inherently unstable, complicating their extraction from LRM. Traditional extraction methods utilizing polar solvents like ethanol, methanol, and hot water are not very inefficient. Enhancements such as ultrasound or microwave assistance have substantially improved yield and efficiency [38]. Innovations like the aqueous two-phase system (ATPS) leverage short-chain alcohol and inorganic salt to optimize hydrophilic compound extraction, marking significant advancements in the field [38]. Environmentally sustainable methods such as supercritical water extraction (SWE), high hydrostatic pressure extraction (HHPE), and hydroxypropyl β-cyclodextrin (HP β-CD) solutions have also surpassed traditional techniques in environmental impact and extraction efficiency [15,39]. For further purification, macroporous resins like AB-8, XDA-6, and D-101 have been effective, with purified anthocyanins showing enhanced thermal stability [38,40,41]. More importantly, emerging methods like semi-continuous liquid-phase pulsed discharge (LPD) and ultrasound-assisted centrifugal extraction (UACE) have demonstrated superior efficiency and reduced energy consumption [5,16]. Researchers have found that combining vacuum freeze drying (VFD) with ultrasonic-assisted enzymolysis extraction (UAEE) significantly boosts yield and antioxidant activity, optimizing extraction for food industry applications [42].

Table 1
LRM bioactive compounds and their bioactive abilities.

Classification	Bioactive compounds	Trial subject	Bioactive abilities	Potential mechanism	References
Flavonoid	Anthocyanins	The DPPH, ABTS, and FRAP assay	Antioxidant activity		[15,17]
Flavonoid	Anthocyanins	Strains (*Staphylococcus aureus*, *Escherichia coli* (*E. coli*), Aspergillus niger and Penicillium sp.)	Antibacterial effect	Damage cell membrane integrity and inhibit biofilm metabolism of food-borne pathogens	[45]
Flavonoid	Anthocyanins and P3G	Fermentation in vitro	Intestinal health promotion	Regulate the gut microbiota of healthy and IBD feces	[11]
Flavonoid	Anthocyanins	Simulated digestion and fermentation in vitro	Intestinal health promotion	Regulate gut microbiota structure, promote SCFAs production	[50]
Flavonoid	P3G (Anthocyanin)	Nematode strains	Anti-aging effect	Promote the nuclear translocation of DAF-16 in *Caenorhabditis elegans*, improve gut microbiota	[20]
LRM extracts	Main compounds: Anthocyanins	C57BL/6J male mice	protection against Acute alcoholic liver disease	Nrf2/HO-1/NF-κB signaling pathway: upregulate Nrf2 and Ho-1,downregulate Nf-κb and Tnf-α genes	[21]
LRM extracts	Main compounds: Anthocyanins and Spermidines	D-galactose D-Gal) aging model mice	Anti-aging effect	Reduce D-Gal-induced oxidative stress: decrease the LPO and MDA, increase SOD and GSH-Px	[78]
LRM extracts	Anthocyanins	Diabetic model of KM mice	Hypoglycaemic activity,	Decrease postprandial blood glucose, inhibit α-glucosidase	[52]
LRM extracts	Delphinidine 3 glucoside	Chronic ocular hypertension model mice	Anti- Glaucoma	Mitigate oxidative stress and reduce microglial activation in the retina	[55]
Flavonoid	Petunidin 3-O-[6-O-(4-O-(trans-p-coumaroyl)-a-L-rhamnopyranosyl)-b-D-glucopyranoside]-5-O-[b-D-glucopyranoside]	PC12 cells in vitro	Antioxidant activity	Scavenge free radicals; recover plasma membrane integrity, decrease MDA production, and increase antioxidant enzymes secretion	[46]
Flavonoid	Anthocyanins	Caco-2 cells; Hep-G2 cells	Diabetes Treatment	Inhibit α-glucosidase activity and alleviate insulin resistance	[12]
Flavonoid	Anthocyanins	Prostate cancer DU-145 cells	Anti-cancer effect	Inhibit cell proliferation and induce apoptosis through the ROS/PTEN/PI3K/Akt/caspase 3 signaling pathway	[47]
Flavonoid	Anthocyanins	Males C57BL/6 mice	Health promotion	Enhance liver antioxidants, reduce colon inflammation, and improve gut barrier and microbiota	[10]
Flavonoid	Anthocyanins	Male C57BL/6J mice	Intestinal health promotion	Modulate the Gut Microbiota, inhibiting the LPS/NF-kB/TLR4 pathway	[103]
Flavonoid	purified anthocyanins	Male SD rats	Atherosclerosis amelioration	Modulate gut microbiota and NF-κB/SREBP-2 pathways	[104]
Flavonoid	anthocyanin extracts and petunidin-3-glu	Male SD rats	Gouty arthritis alleviation	Reduce paw swelling, lower the level of IL-1β, IL-18, COX-1, TNF-α, and prostaglandin E2 in the serum	[105]
Flavonoid	Anthocyanins	Female SD rats	Neuroprotective effects	Alleviate D-Galactose-Induced Memory Impairment, Oxidative Stress, and Neuroinflammation via RAGE/NF-κB/JNK pathway	[106]
Flavonoid	Anthocyanins	Male C57BL/6J mice, Tlr4−/− male mice	Anti-inflammatory effect	Promote Intestinal Barrier Integrity and the Lactobacillus Proliferation to ameliorate High-Fructose Diet-Induced Neuroinflammation	[9]
Flavonoid	Anthocyanins	Male CD-1 mice	Neuroprotective effects	Suppress the MLK3 activation	[107]
Flavonoid	Pn3G5G (Anthocyanin)	Female SD rats	Neuroprotective effects	Alleviate cognitive dysfunction, oxidative stress and neuroinflammation, and shift the abnormal	[54]

(*continued on next page*)

Table 1 (*continued*)

Classification	Bioactive compounds	Trial subject	Bioactive abilities	Potential mechanism	References
				hippocampal metabolites induced by Dgalactose.	
Flavonoid	Anthocyanins	Male ICR mice	Nicotine withdrawal anxiety reduction	Regulate ER receptors directly or regulate the MAPK and PI3K/Akt signaling pathway indirectly	[53]
Flavonoid	Pt3R5G (Anthocyanin)	Male BALB/c-nude mice	Anticancer effects	Inhibit cell proliferation through inducing ferroptosis by down-regulating SLC7A11 in colon cancer	[48]
Flavonoid	Anthocyanins	Synovial fibroblast (SF)	Inhibition for the hyperproliferation of SFs (relating to Rheumatoid arthritis)	Inhibit cell growth, initiate apoptosis, and induce pyroptosis	[8]
Flavonoid	Anthocyanins	Slc25a46−/− mice	Attenuation for mitochondrial dysfunction (relating to aging-related diseases)	Cognition-improving property	[79]
Flavonoid		Male ApoE null mice	Nonalcoholic fatty liver alleviation	Decrease ROS production and inflammation, increase fatty acids oxidation, and reduce fatty acids synthesis	[77]
Flavonoid	Catechin		Antioxidant activity, obesity management		[35]
Flavonoid	Naringenin		Tumor-growth inhibition, anti-atherogenic effect		[35]
Flavonoid	Rutin		Anti-oxidation, neuroprotection, cytoprotection, vasoprotection and cardioprotection		[35]
Phenolic acid	chlorogenic acid		Antioxidant activity, obesity control	Regulate glucose absorption through AMPK activation to control obesity	[35]
Dihydrochalcone flavonoid	Phloretin		Anti-inflammatory effect	Attenuate mucus hypersecretion and airway inflammation and alleviate diabetic neuropathy	[35]
Protocatechuic acid	Protocatechuate		Antioxidant activity, anti-trichophyton		[35]
Phenylpropanoid derivative			Antioxidant activity	DPPH radical scavenging activity, oxygen radical absorbance capacity (ORAC),α-glucosidase inhibitory activity	[75]
Polysaccharide	LRP-S2A	In vitro	Osteoblast differentiation improvement		[67]
Polysaccharide		In vitro	Antioxidant activity	Scavenge DPPH radical, Hydroxyl radical and Superoxide radical	[18]
Polysaccharide	LRGP5	RAW 264.7 cells	Immunomodulatory effect	Promote macrophage proliferation, enhance nitrogen monoxide secretion	[66]
Polysaccharide	LRGP3	RAW 264.8 cells	Immunological activity	Benefit for complement fixating activity expression and macrophage activation activity)	[108]
Polysaccharide	LRGP3	RAW 264.9 cells	Anti-inflammatory effect	Inhibit TLR4/NF-kB signaling pathway to attenuate LPS-induced inflammation	[19]
Polysaccharide	LRP3	SD rats	Neuroprotective effect	Mitigate OGD/R-induced neuronal injury in rat primary cortical neurons.	[13]
Polysaccharide	LRP1-S2	AsPC-1, BxPC-3 pancreatic cancer cell lines, BALB/cA nu/nu mice	Anti-tumor effect	Induce apoptosis of BxPC-3 by inactivating P38 MAPK/NF-κB and GSK-3β/β-Catenin signaling pathways to	[14]

(*continued on next page*)

Table 1 (*continued*)

Classification	Bioactive compounds	Trial subject	Bioactive abilities	Potential mechanism	References
				decrease pancreatic cancer cells proliferation	
Polysaccharide	LRGP3	Kunming female mice (immunosuppressive)	Immunomodulation	Promote immune organs development, lymphocyte proliferation and macrophage phagotytosis, regulate the levels of serum cytokines	[60]
Polysaccharide		Male BALB/c male mice	Anti-fatigue activity	Mobilize triglyceride during exercise and protect corpuscular membrane	[59]
Fatty Acid and Phytosterol	Main compounds: Linoleic, Oleic, 24-methylenecholesterol, Campesterol		Antioxidant activity; enzyme inhibition	Scavenge DPPH/ABTS radical, inhibit pancreaticlipase and cholesterol esterase	[70]
Fatty acid and mineral content	Main compounds: Linoleic, Oleic, Potassium, Calcium, and Magnesium	Mice model	Myocardial protection	Maintain the functional integrity of myocardial tissue	[68]

Table 2
Extraction, purification, and identification for LRM anthocyanins.

Extraction method	Purification	Identification	Major findings	References
28 % $NaH_2PO_4/(NH_4)_2SO_4$-26 % ethanol ATPS, 45.5 °C, 45min	XDA-6	HPLC-ESI-MS/MS, HPLC-DAD	99.84 % anthocyanins recovery, over 70 % of total sugar were removed.	[41]
Ultrasound-ATPE (25%EtOH/20 %$(NH_4)_2SO_4$), 33.7min, 600W, solid: liquid (S: L) = 1:50, pH:3.98	D-101		Yield: 4.71 mg/g dry LRM fruit	[38]
Ultrasonic–80 % alcohol, S: L = 1:40, 50 °C,3h	AB-8	UV–Vis	Applied for qualitative research.	[50]
Ultrasound-90 % ethanol, S: L = 1:5, RT, 1.5h	AB-8	UPLC-Q-Orbitrap MS	Extraction yield: 22.5 %, 5 anthocyanidin and 5 anthocyanin were identified.	[105]
Subcritical water, 170 °C, 55min, 3 mL/min, 6.8 MPa	–	HPLC-MS, UPLC-Triple-TOF/MS	Yield: 26.33 %, 7 anthocyanins were characterized	[15]
EtOH: H_2O = 8:2, S: L = 1:30, 3h, pH 2.5	YMC-Pack ODS-A column	HPLC, MS, NMR	845.6 ± 43.9 mg cyanidin-3 glucoside/100 g DW	[46]
70 % ethanol, 2h, S: L = 1:20, pH:2.5	AB-8	HPLC-DAD/QTOF-MS/MS, UV/Vis	16 anthocyanins were tentatively identified.	[37]
70 % ethanol, 2h, S: L = 1:20, pH:2.5	AB-8	HPLC (XCharge C8SAX column)	Two pairs of cis−trans isomeric anthocyanins and one new anthocyan were identified.	[40]
80 % ethanol (0.5 % HCl), 4 °C, 24h, S: L = 1:2	AB-8	HPLC-MS	6 anthocyanins were identified.	[86]
Methanol (2%FA), S: L = 1:1, dark, RT, 24h	D-101	HPLC-MS	4 major anthocyanins were obtained.	[44]
Methanol (2 % FA), dark, 24h	–	HPLC-DAD HPLC-ESI-MS	14 anthocyanins have been detected, 10 of which were characterized.	[17]
Methanol: FA (98:2), dark, RT 24h	–	HPLC-DAD HPLC-ESI-MS	10 anthocyanins have been identified.	[27]
1.65 % β-cyclodextrin, 50 °C, 30 min, S: L = 1:15	–	UPLC-DAD	Green methods for the analysis of anthocyanins.	[39]
Semi continuous liquid-phase pulsed discharge (LPD) system: ethanol (20 %), pH 7, 8 kV, 27 mL/min, 8 min	–	HPLC-MS	Recovery rate: 95.69 %	[5]
UACE: petroleum ether−ethyl acetate−ethanol−water (2:2:1:6, v/v/v/v)	Countercurrent chromatography	HPLC	The purities of 6 target anthocyanins: over 97.03 %	[16]
Ultrasound with 70 % ethanol for 1.5 h	AB-8	HPLCUPLC–triple-TOF MS/MS	Anthocyanin concentration: 75.99 % ± 0.21 % 10 anthocyanins were identified	[52]

4.1.1.2. Characterization. Anthocyanins are complex molecules consisting of glycosides like petunidin, pelargonidin, and cyanidin combined with monosaccharides such as glucose and arabinose. They are also linked to aromatic carboxylates like p-coumaric acid and coffeic acid [40]. Most anthocyanins in LRM are found to be acylated, affecting their stability and behavior [17]. Petunidin derivatives are predominant in LRM fruits, accounting for 95 % of the total anthocyanin content. The most common of these is petunidin 3-O-rutinoside (trans-p-coumaroyl)-5-O-glucoside, making up nearly 80 % of the total anthocyanins [2,17]. So far, researchers have identified 37 anthocyanins in LRM fruits using advanced techniques like mass spectrometry (MS) and nuclear magnetic resonance (NMR) for accurate structural analysis. However, distinguishing between cis-trans acylated anthocyanin isomers remains challenging due to similar fragmentation patterns in MS [37]. NMR provides an alternative by utilizing distinct coupling constants, requiring sufficiently pure anthocyanin samples [40]. High-performance liquid chromatography (HPLC) with a reversed-phase (RP) column is also an adjunct identification tool. The elution sequence of anthocyanins on the RP column can differentiate between cis and trans configurations [43].

4.1.1.3. Biological activities. LRM anthocyanins are extensively studied for their antioxidant properties, validated by assays like DPPH, ABTS, and FRAP [17]. Their effectiveness is enhanced by structural features such as hydroxyl groups, which significantly boost antioxidant capabilities [44]. Additionally, acylation not only improves their stability but also their antioxidant effectiveness by protecting against nucleophilic attacks. The broader implications of anthocyanins' antioxidant properties include protective effects against environmental stressors and potential antibacterial activities against pathogens like Staphylococcus aureus [45].

Regarding health benefits, anthocyanins may demonstrate a broad spectrum of positive effects. For instance, Petunidin 3-O-[6-O-(4-O-(trans-p-coumaroyl)-α-L-rhamnopyranosyl)-β-D-glucopyranoside]-5-O-[β-D-glucopyranoside] (Pt3G) has shown significant antioxidant activity, effectively scavenging DPPH and ABTS free radicals and offering protection against oxidative damage in neuron-like rat pheochromocytoma (PC12) cells [46]. Similarly, petunidin 3-O-[rhamnopyranosyl-(trans-p-coumaroyl)]-5-O-[β-D-glucopyranoside] (Pt3R5G) exhibited not only strong antioxidant properties but also anti-cancer effect by inhibiting the growth and promoting the death of cancer cells, including those in prostate cancer DU-145 cells through the ROS/PTEN/PI3K/Akt/caspase 3 pathway [47,48]· Additionally, anthocyanin compounds have been implicated in targeting genes related to rheumatoid arthritis pathogenesis and reducing abnormal cell proliferation [8]. Crude anthocyanins and their primary monomer have been demonstrated intestinal anti-inflammatory effects in a dextran sodium sulfate (DSS)-induced colitis model [49]. Their potential to modulate gut microbiota suggests a beneficial role in digestive health, with in vitro studies showing regulation of microbiota composition indicative of their capacity for dietary interventions in gut health maintenance [10,11,50]. Recent study has been shown that P3G from LRM reduces obesity and liver steatosis in high-fat diet-induced obese mice by modulating gut microbiota composition, highlighting its potential for obesity prevention [51]. Similar studies have reported that LRM anthocyanins exhibited their Inhibition for α-glucosidase activity in diabetic mice models and cell models, indicating their potential in the prevention of obesity and diabetes[12,52]. Additional studies highlight the multifaceted health-promoting functions of LRM anthocyanins, including mitigating anxiety associated with nicotine withdrawal and showing promise in cognitive dysfunction treatment and hypertension management [53,54]. Also, a new study demonstrated that LRM P3G markedly extends lifespan, enhances stress endurance, and modulates gut microbiota in Caenorhabditis elegans, highlighting its potential anti-aging properties through the activation of the DAF-16 pathway [20]. Another recent study showed that oral intake of LRM water extract, mainly delphinidin 3 glucoside, could protect retinal ganglion cells from oxidative stress and neuroinflammation in glaucoma models. Through its antioxidative and microglial inhibition effects, this extract potentially offers a preventive and therapeutic strategy [55]. Although these findings suggested LRM anthocyanins and their monomers exhibited diverse health-promotion functions on cell or animal models, more credible clinical evidences need to be found.

4.1.2. Other flavonoid compounds

In addition to its notable anthocyanins, LRM is enriched with a diverse spectrum of flavonoids, including rutin, catechin, naringenin, quercetin, and kaempferol, which collectively enhance its phytochemical profile [35]. Among these, rutin is particularly prominent, found abundantly in natural sources such as passionflower, tea, buckwheat, and apples. It is recognized for its extensive bioactivities, offering antioxidation, neuroprotection, cytoprotection, vasoprotection, and cardioprotection, thus underscoring its critical role in LRM's bioactive matrix [35]. While rutin is distinguished as a principal bioactive constituent of LRM, ongoing research is vital to further substantiate its benefits and applications. Catechin and naringenin are also significant, with catechin known for its robust antioxidant properties and efficacy in managing obesity, and naringenin recognized for its potential anti-tumor and anti-atherogenic effects [35]. These compounds underscore the therapeutic potential of LRM beyond its primary anthocyanin content, suggesting a rich tapestry of health benefits derived from its broader flavonoid profile.

4.1.3. Phenolic acids

Chlorogenic acid is a standout phenolic acid in LRM, with concentrations surpassing those found in other berries from the Tibetan plateau, such as *Nitraria tangutorum* Bobrov, *Hippophae rhamnoides* L., *Lycium barbarum*, and *Rubus corchorifolius* L.f [34,35,56]. Accompanied by significant levels of caffeic, ferulic, and gallic acids, LRM's array of phenolic acids enriches its phytochemical diversity and enhances its functional properties.

Chlorogenic acid is particularly noted for its protective effects against oxidative stress-induced cellular damage and its crucial roles in lipid and glucose metabolism management. The presence of chlorogenic acid in significant quantities has prompted debates on whether the health benefits attributed to LRM are primarily due to this compound alone or arise from synergistic interactions with other components such as rutin [35].

Moreover, these phenolic acids are believed to enhance LRM's antioxidant capabilities through mechanisms like radical scavenging and the reduction of lipid peroxidation. This synergistic action not only bolsters the antioxidant capacity of LRM but also suggests that these phenolic acids play a crucial role in its overall health-promoting properties [57,58]. This highlights the potential for further research and the development of health products based on LRM's phenolic acids, presenting a compelling area for nutritional science and therapeutic applications.

4.2. Polysaccharides

LRM is abundant in a variety of polysaccharides, which play critical roles in its biological functionalities. These polysaccharides are not only key to LRM's structural integrity but also contribute significantly to its health-promoting attributes [13,19,59,60].

4.2.1. Extraction and analysis

Traditional hot-water extraction methods, although safe and straightforward, require high temperatures and prolonged periods, which can degrade the quality of the extracted polysaccharides [61]. By contrast, microwave-assisted and ultrasonic-assisted extraction has been adopted for its efficiency and selectivity [62]. Moreover, apart from physical methods, enzyme-assisted extraction is another choice for extracting polysaccharides from LRM fruit tissues [62].

Effective analytical techniques such as high-performance gel permeation chromatography, methylation analysis, partial acid hydrolysis, gas chromatography, infrared spectroscopy, electrospray ionization mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR) have been utilized to elucidate the complex structures of LRM polysaccharides. To date, research has identified nine branched polysaccharides in LRM, including LRP1, LRP2, LRP3, LRP4, LRP5, LRGP3, LRP-S2A, LRP3-S1, and LRP1-S2, which are primarily composed of arabinose, galactose, glucose, rhamnose, xylose, and minor protein content [14,63–67].

4.2.2. Biological activities and therapeutic potential

LRM polysaccharides exhibit potent antioxidant properties, effectively scavenging various radicals in vitro and demonstrating their potential as natural antioxidants suitable for food applications [18]. Beyond antioxidation, these polysaccharides also display anti-fatigue effects, enhancing lipid metabolism and protecting cellular membranes during physical exertion in mouse models [59].

LRGP3, a highly branched polysaccharide, has shown notable anti-inflammatory effects in mouse macrophage RAW264.7 cells by inhibiting the Toll-like receptor 4/NF-κB (TLR4/NF-κB) signaling pathway, resulting in reduced proinflammatory cytokines and iNOS mRNA expression [19]. It also supports immune response by enhancing spleen and thymus health and promoting B and T cell proliferation. Similarly, LRP3 is particularly promising for its role in mitigating neuronal injury under conditions of oxygen-glucose deprivation/reoxygenation, potentially addressing neonatal hypoxic-ischemic encephalopathy through the activation of the Nrf2/HO-1 signaling pathway [13]. By contrast, LRP5, an immunologically active pectin, serves as an immunomodulator, encouraging macrophage proliferation and boosting nitrogen monoxide secretion. The structural diversity of LRM polysaccharides underpins their vast therapeutic potential, with applications ranging from osteoblast differentiation to inhibiting pancreatic cancer cell proliferation and inducing apoptosis in targeted cancer cells with minimal cytotoxicity to normal cells [14,63].

4.3. Fatty acid and phytosterols

LRM oils are distinguished by their rich content of essential fatty acids, including linoleic, oleic, palmitic, linolenic, and stearic acids, which are crucial for various biological functions [68]. The seeds of LRM, constituting about 30 % of the fruit's dried weight during processing, are particularly high in polyunsaturated fatty acids, accounting for 63.99 % of the seed oil [69]· Linoleic acid, an ω-6 polyunsaturated fatty acid, is noted for its extensive health benefits, providing protection against conditions like oxidation, hyperglycemia, hyperlipidemia, thrombosis, and cancer [70].

Despite the recognized health benefits of these fatty acids, research into their specific biological impacts from LRM remains limited. Zhao et al. have notably reported on the seed oils' antioxidant capabilities, as demonstrated in assays such as DHHP, ABTS, and FRAP, and their effectiveness in inhibiting lipolytic enzymes like pancreatic lipase and cholesterol esterase (CEase) [70]. These preliminary findings underscore the need for more comprehensive research to elucidate the health-promoting actions and underlying mechanisms of LRM fatty acids.

In addition to fatty acids, LRM seed oils are rich in phytosterols, a group of unsaponifiable compounds known for their significant health benefits. These include 24-methylene cholesterol, campesterol, stigmasterol, dihydro lanosterol, 24-methyl desmosterol, β-sitosterol, Δ5-avenasterol, cycloartenol, and Δ7-avenasterol [70]. Phytosterols have shown potential in mitigating inflammation and diabetes, underscoring their beneficial role in dietary applications [70].

4.4. Carotenoids

In the green fruit stage of LRM, the predominant carotenoids are chloroplastic, including violaxanthin, lutein, and β-carotene [71]. These compounds play crucial roles in the plant, with β-carotene, α-carotene, and β-cryptoxanthin essential for vitamin A biosynthesis. Lutein and zeaxanthin are expressly noted for their potential to mitigate age-related retinal damage, underscoring their importance in ocular health [71].

However, the progression from immature to ripe fruit sees a marked decrease in these chloroplastic carotenoids, culminating in levels too low to detect in mature LRM fruit [33,71]. This reduction is attributed to the absence of chromoplast biogenesis, leading to diminished carotenoid biosynthesis and enhanced degradation. Specifically, the upregulation of carotenoid cleavage dioxygenase 4 is implicated in this decline, resulting in the near absence of detectable carotenoids in ripe LRM fruit[71]].

This phenomenon highlights a significant shift in carotenoid composition and concentration throughout the maturation of LRM, pointing to a complex interplay of biosynthetic and degradative processes that regulate carotenoid levels.

4.5. Alkaloids

Within the chemical profile of LRM, hydroxycinnamic acid amides (HAAs) are notable alkaloids. These compounds are characterized by N-acylated biogenic amines connected to hydroxycinnamic acids through amide bonds. Distinctive HAAs such as *N, N*-bis-dihydrocaffeoyl spermine, *N, N*-bis-dihydrocaffeoyl-spermidine, and dihydrocaffeoyl-caffeoyl-spermidine derivatives are pivotal in differentiating LRM from other species within the *Lycium* genus [72]. The presence of HAAs not only contributes to the plant's defense mechanisms against pathogens but also showcases a range of biological activities. Specifically, these compounds, including various polyamines classified as HAAs, have demonstrated significant anti-inflammatory properties and the ability to inhibit α-glucosidase and cytotoxic activities, suggesting potential therapeutic applications [73].

4.6. *Other compounds*

In the diverse phytochemical landscape of LRM, two coumarins (scopoletin and sculetin) and a cinnamate derivative (Methyl-2-hydroxy-4-undecanoxy-trans-cinnamate) have been identified and extracted [74]. Coumarins, known for their antioxidant properties and enzyme inhibition capabilities, find utility as functional additives in food and cosmetic formulations.

Further research has unveiled the presence of 26 phenylpropanoid derivatives within LRM fruits, with some exhibiting pronounced DPPH radical scavenging activity and oxygen radical absorbance capacity. Notably, a specific phenylpropanoid derivative demonstrated α-glucosidase inhibitory activity, rivaling acarbose's, suggesting its potential in managing glucose metabolism [75].

Additionally, exploration into the endophytic fungi associated with LRM has led to the discovery of four pyrone derivatives from *Aspergillus tubingensis*: 6-isovaleryl-4-methoxy-pyran-2-one, rubrofusarin B, asperpyrones A, and campyrone A [76]. Among these, rubrofusarin B has shown significant inhibitory effects against *Escherichia coli*, highlighting the antibacterial potential of pyrone compounds derived from LRM's endophytic fungi.

LRM is also characterized by its mineral content, comprising essential nutrients such as potassium, calcium, magnesium, and trace elements, including copper, iron, manganese, and zinc [68].

5. Functional properties and applications

Bioactive extracts from LRM have shown efficacy in reducing inflammation and oxidative stress markers in ApoE−/− mice on a cholesterol-enriched high-fat diet [77]. LRM ethanolic extract has been shown to mitigate oxidative stress and improve cognitive function in D-galactose-induced aging model mice, highlighting its potential as a healthcare product for treating age-related diseases [78]. Additionally, LRM extract has alleviated ethanol-induced acute alcoholic liver disease (ALD) in mice by reducing oxidative damage and inflammation through modulation of the Nrf2/HO-1/NF-κB pathway, demonstrating the potential for ALD prophylaxis and treatment [21].

In the context of neurodegenerative disorders, the Slc25a46−/− murine model has revealed the efficacy of LRM extract in improving mitochondrial dysfunction, enhancing muscle strength, and increasing body mass, suggesting potential for Alzheimer's disease intervention [79]. Preliminary clinical studies also suggest that daily intake of LRM might influence the progression of Parkinson's disease by affecting metabolic pathways involved in glycerophospholipid and α-linolenic acid metabolism. However, conclusive statistical significance has yet to be established [80].

LRM supplementation has induced beneficial shifts in gut microbial populations in a C57BL/6 murine model, enhancing intestinal barrier function, modulating microbial diversity, and increasing short-chain fatty acid (SCFA) production. This highlights its role in nutrition, metabolism, pathogen resistance, and immune regulation [81].

Traditionally, LRM has been used in Chinese medicine to treat various ailments, including menopausal symptoms, abnormal menstruation, cardiovascular disorders, and digestive issues, with its roots and bark also used for treating urolithiasis and gingival bleeding [63,82]. A comparative study has indicated that LRM possesses superior anti-inflammatory and antioxidant properties compared to L. barbarum during in vitro gastrointestinal digestion, suggesting significant potential for further research and application in the food industry [7].

The diverse biological components of LRM establish it as a valuable functional food within the Chinese market and beyond. Despite the consistent presence of dietary fibers, soluble sugars, organic acids, minerals, and vitamin C in LRM fruits, the levels of functional components like polyphenols, phenolic acids, flavonoids, and polysaccharides vary considerably across different regions.

The commercial landscape for LRM, primarily in dried fruits, reflects a broad price range indicative of its valued status and the agricultural interest it has aroused, particularly among farmers in northwest China. Despite the economic potential, challenges such as lack of product diversification and variable quality have impacted profitability. Recent research suggests that processing methods, especially superfine grinding, significantly influence the physicochemical properties and antioxidant effectiveness of LRM products, underscoring the need for innovative processing techniques to enhance their economic viability [83].

An expanding range of deep-processed LRM products has broadened its commercial presence. LRM-based kombucha, for instance, offers increased phenolic content and antioxidant activity along with preferred sensory properties compared to traditional kombucha, emphasizing LRM's potential as a functional ingredient in beverage production [84].

Beyond nutritional and health applications, LRM's utility spans food preservation, medical appliances, and textile processing. The rising consumer interest in natural colorants, driven by concerns over synthetic dyes' potential behavioral impacts on children with attention deficit hyperactivity disorder (ADHD), has spotlighted petunidin-3-trans-p-coumaroyl-rutinoside-5-glucoside. This compound's water solubility and stable, vibrant coloration across various pH levels align with the increasing demand for natural and 'clean label' products, offering applications in pH-sensitive food freshness indicators [85]. Innovative applications include the use of Al^{3+} complexes to enhance the color stability of LRM extract in hydrogels for CO_2-sensitive freshness monitoring of chicken and κ-carrageenan and LRM extract-based films (Car-LRM) for milk and shrimp freshness, offering low toxicity alongside antioxidant benefits [22, 24]. Cassava starch-LRA films exhibit remarkable light-blocking, antioxidant, and pH-responsive properties, enhancing the preservation of pork freshness [86]. Moreover, films made from sodium alginate-konjac glucomannan (SA-KGM) infused with LRM anthocyanins and tea polyphenols extend the shelf life of meat by 2–4 days at 4 °C [26] and efficiently track milk freshness[23] Starch/polyvinyl alcohol (SP) films incorporating anthocyanin-loaded nanocomplexes versus free anthocyanins show differences in moisture blocking, mechanical strength, and light blocking, indicating their suitability for extending the shelf life of refrigerated foods like largemouth bass fillets [25] Other research suggested that LRM anthocyanins, at a concentration of 2 g/100 mL, could be an excellent natural purple colorant in yogurt and fermented milk, providing excellent color stability and antioxidant activity during storage [87]. Researchers have been working on developing more sensitive active packaging. A smart film consisting of myofibrillar protein, bacterial nanocellulose, and LRM anthocyanin effectively monitored the real-time freshness of blunt snout bream during storage [88]. Apart from these, research has shown that combining nisin and polylysine as bio-preservatives can extend the shelf life of tender coconut water to 20 days. This result might inspire researchers to explore the potential of LRM extract, another promising bio-preservative, for preserving plant-based beverages [89].

For other aspects, the inclusion of LRM polyphenol in the dough, particularly after liquid fermentation, not only reduces bread digestibility but also improves quality [90]. Furthermore, LRM extracts have found application in imparting antioxidant and antimicrobial properties to wool fabrics, offering a sustainable alternative to conventional textile processing chemicals [91]. The development of fluorescent carbon dots from LRM for bone regeneration underscores its versatility as a natural resource for biomedical applications [92]. Despite positive results for LRM's biological and functional properties, the field remains working to elucidate its mechanisms and innovative application avenues further.

6. Discussion

The study of LRM has garnered significant attention due to its rich bioactive properties and potential applications in nutraceuticals, food processing, and preservation. Research has rigorously characterized significant components such as anthocyanins and polysaccharides, establishing a solid foundation for understanding their therapeutic and nutritional values. This groundwork has positioned LRM as a promising candidate for developing health-promoting food products.

Strengthening Traceability and Authenticity: The traceability of LRM's geographical origin is crucial for ensuring the authenticity and quality of LRM-based products. HPLC-MS [31] can accurately measure the LRM anthocyanin composition, but its destructive detection process is very cumbersome, and the detection cost is not low. Although the electronic nose and tongue [30] can realize non-destructive detection and the detection process is simple, their detection principle determines low sensitivity. Their identification accuracy needs to be strengthened.

The integration of near-infrared hyperspectral imaging and deep learning methodologies has enhanced the rapidity and accuracy of these assessments, marking a significant advance toward non-destructive quality control [93–96]. However, the field still needs to grapple with the challenge of developing cost-effective, user-friendly instruments capable of detecting geographical nuances in LRM's chemical composition, essential for combating fraudulent practices. Regarding the operability of the technology, we believe that improving the sensitivity of the electronic nose and electronic tongue or even using an AI literacy model to trace the origin of LRM is a feasible development direction.

Expanding Bioactivity Research: Regarding the research on the active substances of black wolfberry, its extraction and purification results will directly affect its physiological activity. It should first be ensured that its structure is damaged as little as possible in the extraction and purification steps. In addition to the current standard extraction methods with water or organic solvents [37,40], ultrasound-assisted extraction [38], supercritical fluid extraction [15], or enzyme-assisted extraction techniques [62] have greatly improved the efficiency. As for purification methods, membrane separation techniques and chromatography [16,38,40,41] are currently the most commonly used. However, subsequent studies could use molecularly imprinted polymers, particularly suitable for separating certain structurally complex compounds [97,98]. While the bioactivity of LRM's anthocyanins and polysaccharides is well-documented, their gastrointestinal digestion and bioavailability remain underexplored.

Enhancing the bioavailability of these compounds [99] through innovative encapsulation [100,101] or protein-binding strategies [102] could significantly amplify their nutraceutical value. Moreover, comprehensive in vivo studies and clinical trials are required to validate these findings and facilitate their application in health supplementation.

Innovative Applications: The potential applications of LRM extend beyond traditional uses, opening new avenues in active packaging [23,26], drug delivery systems [92], and cosmeceuticals [91]. For example, developing LRM-infused active packaging could revolutionize food preservation by integrating natural antioxidants that extend shelf life while maintaining food safety. Additionally, exploring LRM's lesser-known compounds, such as organic acids and alkaloids, may uncover new pathways for health innovation, enriching the diversity of applications derived from this versatile plant.

7. Conclusion

This paper has comprehensively elucidated the advancements in the geographical origin tracing of LRM, the progress in its bioactive compounds' extraction, identification, and biological activities, and the research endeavors surrounding LRM in dietary supplements, functional foods, and food preservation. Our review highlights that the techniques for tracing LRM's geographical origin are expected to evolve towards greater precision, simplicity, and rapidity, facilitated by advancements in analytical methodologies and technology.

The investigation of LRM's bioactive constituents has primarily focused on anthocyanins and polysaccharides, demonstrating significant antioxidant, anti-inflammatory, and antimicrobial properties. However, it is essential to broaden the scope of research to include other potentially noteworthy bioactive compounds such as carotenoids, alkaloids, and phenolic acids. Future research should prioritize enhancing the bioavailability and stability of these compounds through innovative extraction and encapsulation techniques. Moreover, the applications of LRM extend beyond functional foods. There is considerable potential for its use in active packaging and food preservation, leveraging its natural preservative properties. Additionally, the pharmaceutical industry may benefit from the therapeutic potential of LRM's bioactive compounds, necessitating further investigation into their mechanisms and efficacy.

In summary, future research will expand LRM's applications, uncovering its unique roles across various industries. By addressing the current gaps in our understanding and exploring innovative applications, we can fully harness LRM's economic and health benefits, contributing to advancements in food technology, nutrition, and medicine.

CRediT authorship contribution statement

Fang Li: Writing – review & editing, Writing – original draft, Software, Investigation, Conceptualization. **Hongjun Li:** Resources, Conceptualization. **Shaobo Li:** Writing – review & editing, Software. **Zhifei He:** Writing – review & editing, Supervision, Conceptualization.

Ethics statement

As it is a review article, ethical approval is not needed.

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Declaration of competing interest

There are no conflicts of interest to declare.

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