

Comparative pharmacodynamic effects of processed *Ligustri Lucidi Fructus* against postmenopausal osteoporosis and potential mechanisms associated with the wine-steamed preparation: Integrating multidimensional pharmacodynamics, serum chemistry, and spatial metabolomics

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Article

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Abstract

Ligustri Lucidi Fructus (LLF), a traditional Chinese medicine widely used for osteoporosis-related disorders, undergoes processing procedures that may alter its chemical composition and pharmacological properties. However, the comparative pharmacological profiles of different processed LLF preparations and the potential mechanisms associated with processing-enhanced effects remain insufficiently understood. In this study, five LLF preparations, including raw, steamed, wine-steamed, vinegar-steamed, and salt-steamed LLF, were systematically evaluated in ovariectomized (OVX) rats through an integrated strategy combining multidimensional pharmacodynamic assessment, serum pharmacochemistry, spectrum–efficacy correlation analysis, and desorption electrospray ionization mass spectrometry imaging (DESI-MSI)-based spatial metabolomics. Among the tested preparations, wine-steamed LLF (JLLF) exhibited the most comprehensive improvements in bone biomechanical properties, bone turnover markers, inflammatory responses, lipid metabolism, and marrow adiposity under the experimental conditions. Serum pharmacochemistry analysis identified 129 serum-exposed components, and 25 JLLF-characteristic candidate constituents, including 16 prototype compounds and 9 phase I/II metabolites, were prioritized through integrated component–efficacy analyses. DESI-MSI revealed distinct spatial distributions of JLLF-derived constituents in renal tissues and demonstrated that JLLF treatment was associated with the modulation of OVX-induced alterations in renal tryptophan metabolism and secondary bile acid biosynthesis. Collectively, this study provides comparative evidence regarding the pharmacological differences among processed LLF preparations and identifies candidate constituents and metabolic alterations associated with the enhanced effects of wine-steaming processing against postmenopausal osteoporosis.

1. Introduction

Postmenopausal osteoporosis (PMOP), a systemic metabolic bone disorder underpinned by estrogen-deficiency-mediated dysregulation of osteoblast-osteoclast coupling^{1,2}, imposes substantial morbidity and socioeconomic burdens, with current interventions attenuating bone loss yet failing to fundamentally reverse microarchitectural deterioration while predisposing to adverse sequelae^{3,4}. In traditional Chinese medicine (TCM), *Ligustri Lucidi Fructus* (LLF), the dried mature fruit of *Ligustrum lucidum* W.T. Aiton, is used to tonify the liver and kidney and to treat liver-kidney yin deficiency-related manifestations, especially soreness and weakness of the waist and knees and bone-steaming fever. These bone- and kidney-related traditional indications provide a clear ethnopharmacological rationale for investigating LLF against PMOP, which is commonly interpreted in TCM as being closely associated with kidney deficiency and impaired bone nourishment^{5,6}. Contemporary phytochemical investigations have substantiated that LLF is abundantly endowed with triterpenes, phenylethanoid glycosides, iridoids, and flavonoids⁷, which activate Wnt/ β -catenin and PI3K/Akt signaling to promote osteoblastic differentiation, and ameliorate oxidative stress and chronic inflammation⁸, thereby exerting osteoprotective effects and positioning LLF as a potentially advantageous therapeutic agent for PMOP intervention.

The processing of TCM is fundamental to achieving meridian tropism, improving flavor, reducing toxicity, and enhancing efficacy. This is accomplished through controlled heating, the use of adjuvants, and process optimization⁹, which are integral components of the technology and theoretical foundation of TCM. Modern standardized preparations of LLF include raw (SLLF), steamed (QLLF), wine-steamed (JLLF), vinegar-steamed (CLLF), and salt-steamed (YLLF) products, each leading to distinct compositional changes and corresponding bioactivity profiles¹⁰. Wine-steaming enhances the solubility of specnuezhenide and oleuropein, thereby increasing antioxidant activity^{11–13}, while salt-steaming enriches oleanolic acid, thereby enhancing osteoblast alkaline phosphatase (ALP) activity and mineralization¹⁴. Notwithstanding these mechanistic insights, anti-osteoporotic formulations incorporating LLF exhibit considerable heterogeneity in processing selection, which significantly limits their evidence-based clinical application. The pharmacodynamic differences among these processed products in relation to PMOP remain unresolved, primarily due to the complex pathophysiology of PMOP. This complexity involves bone metabolic dysregulation, disturbances in the inflammatory microenvironment, and oxidative stress-induced skeletal deterioration^{15–17}. The multifaceted nature of PMOP challenges the efficacy of unidimensional evaluative frameworks, which are insufficient for a comprehensive understanding of the differential therapeutic profiles of processed products. These frameworks fall short in assessing their impact on bone quality improvement, modulation of turnover imbalances, and restoration of microstructural integrity. Furthermore, they do not adequately identify which specific constituents are most influential in mediating distinct therapeutic effects.

The pharmacodynamic divergence is fundamentally linked to compositional changes, structural transformations, and the quantitative redistribution of chemical constituents induced by processing. However, not all chemical components are effectively absorbed and bioavailable to exert osteoprotective effects. Strategies based on serum pharmacochimistry, in conjunction with spectrum-efficacy correlation analysis, support the prioritization of serum-detected constituents associated with pharmacodynamic outcomes. This approach shifts the focus of substance basis investigation from total ex vivo constituents to in vivo bioavailable components^{18–20}. Nonetheless, establishing quantitative structure-efficacy relationships between absorbed constituents and multidimensional pharmacodynamic endpoints remains a significant challenge in elucidating the material substrate of processing-enhanced efficacy. In particular, the tissue distribution and local metabolic regulatory effects of bioactive constituents at target organs remain poorly characterized. According to the TCM theory of “the kidney governing bones and marrow”, renal metabolic homeostasis is closely coupled with skeletal health, and disordered renal metabolism is an important pathological link in the development of PMOP²¹. Desorption electrospray ionization mass spectrometry imaging (DESI-MSI) enables in situ visualization of the spatial distribution of exogenous components and endogenous metabolites in tissues with high spatial resolution, providing a powerful tool for dissecting the regional metabolic regulatory effects of TCM at the tissue level^{22–25}. The integration of serum pharmacochimistry and spatial metabolomics provides a robust technical foundation for systematically analyzing the substance basis and tissue-level mechanisms through which differentially processed LLFs exert anti-PMOP effects.

The present study employed the ovariectomized (OVX) rat as a PMOP experimental model²⁶, integrating multidimensional pharmacodynamic evaluation, serum pharmacochemistry, spectrum-efficacy correlation analysis, and DESI-MSI spatial metabolomics (Fig. 1). Pharmacodynamic differences among five LLF preparations (SLLF, QLLF, JLLF, CLLF, YLLF) were systematically compared to identify processing-potentiated bioactive constituents, and to explore the spatial metabolic regulatory effects of JLLF on renal tissues, so as to elucidate the processing efficacy enhancement mechanism of LLF against PMOP from the perspective of kidney-bone axis regulation.

2. Materials and Methods

2.1. Materials and chemicals

Dried mature fruits of *Ligustrum lucidum* W.T. Aiton (*Ligustri Lucidi Fructus*, Nüzhenzi; batch No. C24081911) were produced in Pengzhou, Sichuan Province, China, and purchased from Anguo Runde Pharmaceutical Co., Ltd. (Anguo, Hebei, China), and authenticated by thin-layer chromatography per the Chinese Pharmacopoeia (2025 edition) 6-55-9 (Chinese Pharmacopoeia Commission, 2025). Adjuvants for processing included rice wine (15% v/v ethanol), rice vinegar (≥ 4.5 g/100 mL total acidity), and refined salt. Alendronate sodium tablets (CSPC Ouyi Pharmaceutical Co., Ltd., Shijiazhuang, China) served as the positive control. Sodium chloride, penicillin sodium, and 4% paraformaldehyde (Biosharp Biotechnology, Hefei, China) were used as supplied. All reference standards were HPLC-grade with $\geq 98\%$ purity. Hydroxytyrosol, salidroside, loganin, echinacoside, and betulin were purchased from Shanghai YuanYe Biotechnology Co., Ltd. Oleuropein, nuezhenoside G13, oleanolic acid, rutin, hyperoside, apigenin 7-O-glucoside, tormentic acid, verbascoside, apigenin, ligustroflavone, specnuezhenide, luteolin, loganic acid, oleoside, and nobiletin were obtained from Chengdu Manst Biotechnology Co., Ltd. Verbenalin was sourced from Chengdu Kroma Biotechnology Co., Ltd.; taxifolin and schizandrin A were procured from the National Institutes for Food and Drug Control. HPLC-grade methanol and acetonitrile and LC-MS-grade formic acid were acquired from Fisher Scientific. Ultrapure water was generated using a Milli-Q purification system (MilliporeSigma). ELISA kits for osteocalcin (OCN), ALP, BALP, IL-10, IL-17, TGF- β , TNF- α , and deoxyypyridinoline (DPD) were purchased from Wuhan EIAab Science Co., Ltd. Colorimetric kits for TC, TG, LDL-C, and HDL-C were obtained from Shenzhen Mindray Animal Medical Technology Co., Ltd. All kits were stored at -20°C and used before their expiration dates according to the manufacturers' instructions.

2.2. Preparation of processed *L. lucidum* fruit samples

SLLF samples were prepared by cleaning, washing, and drying in a ventilated oven at 45°C to constant mass. For the other processed samples, SLLF was mixed with water, rice wine, rice vinegar, or 10% saline (w/w, herb:adjuvant = 5:1), incubated for 24 h, and then steamed for 8 h at 100°C under atmospheric pressure. The resulting materials were spread in a single layer and dried in a ventilated oven at 45°C to constant mass^{6,27}. Residual moisture was determined by loss on drying at 105°C to constant mass, and each batch was required to contain no more than 13.0% moisture. Processing yield (%) was calculated

as $100 \times$ the dry mass of the processed product divided by the dry mass of the starting SLLF. Moisture content and processing yield were recorded as batch quality-control parameters. For oral administration, each sample was extracted twice with 10 volumes of water (v/w, 1 h per extraction). The combined aqueous extracts were concentrated under reduced pressure and lyophilized to yield dry powders.

2.3. Ovariectomy animal model, randomization, grouping, and treatment

SPF-grade 8-week-old female Wistar rats weighing 200 ± 20 g were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. The animal quality certificate number was SCXK (Beijing) 2021-0006, and the animal use license number was SYXK (Beijing) 2023-0011. The animals were housed in the Laboratory Animal Center of Beijing University of Chinese Medicine under SPF-grade barrier conditions. The animal room was maintained at 25°C with a relative humidity of 40%-70% and a 12 h light/12 h dark cycle. All animal experiments were approved by the Experimental Animal Ethics Committee of Beijing University of Chinese Medicine (approval No. BUCM-2024080201-3070).

Ninety-six rats were acclimatized for 7 days. The animals were ranked by body weight, and a sequence generated using the random-number function in SPSS 16.0 was used to allocate them to Sham ($n = 12$) or OVX ($n = 84$) groups. Bilateral ovariectomy was performed as detailed in **(Figure S1)**. Under sodium pentobarbital anesthesia ($40 \text{ mg} \cdot \text{kg}^{-1}$, i.p.), a 1-cm dorsal incision was made 1 cm caudal to the costal arch and 1 cm lateral to the vertebral column; ovaries were ligated with 3-0 silk suture and excised, while uterine horns were preserved^{28,29} (Han et al., 2026; Huang L. et al., 2025). Sham controls underwent identical surgery—only periovarian fat was removed. Penicillin sodium was administered for 3 days postoperatively. Eight weeks later, OVX rats were stratified by body weight and allocated in blocks of seven according to a new computer-generated random sequence into seven groups ($n = 12$): OVX control, alendronate sodium (AS; $1.11 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), and five LLF formulations (SLLF, QLLF, JLLF, CLLF, YLLF; $1.26 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$). The AS dose was selected as an established oral positive-control dose for OVX-rat osteoporosis studies. The administered dose of $1.11 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ approximates the commonly used oral dose of $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in this model and is consistent with body-surface-area scaling of the standard clinical 10 mg/day alendronate regimen^{30,31}. Sham and OVX control groups received 0.5% CMC-Na solution. Before the formal comparative experiment, a preliminary dose-ranging experiment was conducted using SLLF at low, medium, and high doses of 0.315, 0.63, and $1.26 \text{ g} \text{ crude drug} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, respectively. The high dose was calculated from the maximum daily dose recommended in the Chinese Pharmacopoeia (12 g/day for a 60 kg adult) after body surface area conversion and showed the best anti-PMOP effect; therefore, $1.26 \text{ g} \text{ crude drug} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ was selected for the subsequent comparison of different processed LLF preparations^{6,30}.

2.4. Biological sample collection

After a 12 h fast, rats were placed in metabolic cages with free access to water for 24-h urine collection prior to euthanasia. One hour after the final treatment, rats were deeply anesthetized with sodium pentobarbital, and blood samples were collected via abdominal aortic puncture. Subsequently, the

animals were euthanized by exsanguination under deep anesthesia. At the time of sacrifice, the rats were approximately 29 weeks old and weighed 350 ± 30 g. Major organs including heart, liver, spleen, lung, kidney, uterus, and brain were rapidly excised, rinsed with ice-cold saline, gently blotted dry, weighed, snap-frozen in liquid nitrogen, and stored at -80°C . Serum was separated by centrifugation at 3000 g for 15 min at 4°C following 1 h of clotting at room temperature, then aliquoted and stored at -80°C . Major organs including heart, liver, spleen, lung, kidney, uterus, and brain were rapidly excised, rinsed with ice-cold saline, gently blotted dry, weighed, snap-frozen in liquid nitrogen, and stored at -80°C . Bilateral femora and tibiae were dissected, carefully cleaned of adherent soft tissue, and stored under distinct conditions: femora in 4% paraformaldehyde for histomorphometric analysis; tibiae in 4°C PBS for subsequent biochemical and molecular assays.

2.5. Pharmacodynamic evaluation

Animal activity was monitored daily, and body weight was recorded weekly. At necropsy, organ indices were calculated as the ratio of organ weight to body weight multiplied by 100%. For histological analysis, femora were fixed in 4% paraformaldehyde, decalcified in 20% EDTA for 4 weeks, and embedded in paraffin. Serial sections ($5\text{ }\mu\text{m}$) were stained with H&E, Masson's trichrome, and TRAP for examination by light microscopy. Biomechanical properties were assessed using three-point bending tests on left tibiae with a CMT2103 universal testing machine. Parameters including maximum load, fracture displacement, stress, strain, elastic modulus, and stiffness coefficient were recorded with standardized specimen orientation to ensure reproducibility. Right femora were subjected to micro-CT analysis using a SkyScan 1176 system (Bruker, Kontich, Belgium) at 90 kV, 50 μA , and $30\text{ }\mu\text{m}$ resolution. The distal metaphysis was selected as the region of interest (1.8 mm thick, 60 slices commencing 30 slices below the growth plate). Trabecular and cortical parameters were quantified using CT-An software according to ASBMR nomenclature, with all measurements performed by a single operator in a blinded manner. Biochemical assays were conducted using ELISA kits for serum ALP, BALP, OCN, TGF- β , IL-10, IL-17, TNF- α , and urinary DPD. Serum lipid profiles (TC, TG, LDL-C, HDL-C) were determined using an automated biochemical analyzer.

2.6. Serum sample preparation

A 200 μL aliquot of serum was mixed with 800 μL methanol-acetonitrile (1:1, v/v) containing isotope-labeled internal standards (palmitic acid- d_{31} , cholic acid- d_4 , benzoate- d_5 , 100 ng/mL each) in a 1.5 mL Eppendorf tube. The mixture was vortexed for 5 min and sonicated in an ice-water bath for 10 min, followed by centrifugation at $13,200 \times g$ for 15 min at 4°C . The supernatant was dried under vacuum, reconstituted in 100 μL methanol, and centrifuged at $13,200 \times g$ for 15 min at 4°C . The final supernatant was subjected to LC-MS analysis.

2.7. Chromatographic-mass spectrometric conditions and data analysis

Chromatographic separation was performed on an Accucore AQ column ($150\text{ mm} \times 2.1\text{ mm}$, $2.6\text{ }\mu\text{m}$) with a mobile phase consisting of acetonitrile (A) and 0.1% formic acid in water (B). The gradient elution

program was as follows: 0–3 min, 95%→75% B; 3–8 min, 75%→30% B; 8–13 min, 30%→20% B; 13–17 min, 20%→15% B; 17–19 min, 15%→10% B; 19–20 min, 10% B; 20–21 min, 10%→0% B. The flow rate was 0.2 mL/min, the injection volume was 5 μ L, and the column temperature was 35°C.

Mass spectrometry was conducted using an Orbitrap Fusion Lumos Tribrid mass spectrometer with an ESI source. Data-dependent acquisition in full MS-ddMS² mode was used for both positive and negative ions. Key parameters included: sheath gas at 35 Arb, auxiliary gas at 7 Arb, spray voltage at 3.50 kV (+)/3.00 kV (–), ion transfer tube temperature at 300°C, and auxiliary gas heater at 275°C. MS¹ scans had a resolution of 60,000 (m/z 100–1200), and MS² scans had a resolution of 30,000 (m/z 80–1000). HCD collision energies were 20%, 40%, and 60% with a 1.0 Da isolation window and 15 s dynamic exclusion.

2.8. Serum chemical profile-pharmacodynamic correlation analysis

Pharmacodynamic indicators were classified into seven categories: organ indices, histology, bone microarchitecture, biomechanics, bone turnover markers, inflammatory factors, and lipid profiles. The entropy weight method was applied to generate composite efficacy scores by objectively determining indicator weights based on intra-group dispersion, thereby minimizing subjective bias. This methodology is particularly well-suited for comprehensive multi-indicator evaluations in TCM research. Data were preprocessed as inhibition or promotion rates to standardize directional correlations across variables. Serum chemical profiles were normalized using the mean value method for dimensionless processing. Pearson correlation, partial least squares regression (PLSR), and grey relational analysis (GRA) were employed to associate metabolic fingerprints with composite efficacy. Individual rats served as independent biological observations in the correlation analyses. Key bioactive components were identified by a grey correlation degree ≥ 0.8 or Pearson $|r| > 0.7$ with $q < 0.05$.

2.9. DESI-MSI analysis

DESI-MSI was conducted on a SYNAPT XS mass spectrometer coupled with a DESI source (Waters Corporation)³². Mass spectrometric data were collected in both positive and negative ionization modes at a mass resolution of approximately 40,000 (FWHM), with a spatial resolution of 100 μ m. The sample stage was operated at a scanning velocity of 200 μ m/s. The ion source and transfer line temperatures were maintained at 150°C and 250°C, respectively. A spray solution containing 98% acetonitrile and 0.1% aqueous formic acid was used, with leucine enkephalin (200 pg/ μ L) added as the lock mass reference. The spray voltages were set to +1.5 kV in positive ion mode and –1.2 kV in negative ion mode, and ions were detected over an m/z range of 100–1200. After data acquisition, the raw DESI-MS datasets were processed using High-Definition Imaging (HDI) software (Waters) for mass calibration, peak extraction, and signal normalization, followed by visualization of the spatial localization of target metabolites within the kidney tissue.

2.10. Data analysis

Pharmacodynamic data were analyzed using SPSS software (version 16.0, IBM Corp., Armonk, NY, USA). All quantitative data were expressed as mean $\pm$ standard deviation (SD). Data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene’s test before statistical analysis. Differences among multiple groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey’s honestly significant difference (HSD) test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant. In the figures, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ indicate comparisons with the Sham group, whereas $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ indicate comparisons with the OVX group.

LC-MS data were processed using Xcalibur software. Serum-derived prototype compounds and metabolites of LLF were identified by subtracting blank controls to eliminate endogenous interference and by comparison with previously reported chemical profiles of the five processed LLF preparations. Prototype compounds were annotated using MS-DIAL, Compound Discoverer, MS Finder, and PCDL software based on the HMDB, PubChem, DrugBank, Metlin, and mzCloud databases. Metabolites were characterized according to diagnostic fragment ions and metabolic transformation patterns of prototype compounds.

For chemometric analysis, peak areas were extracted using Xcalibur software, corrected using internal standards, and normalized. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed using SIMCA 14 (Umetrics, Umeå, Sweden) to evaluate the overall differences among the five processed LLF preparations. Orthogonal partial least squares discriminant analysis (OPLS-DA) was subsequently performed to identify differential components between JLLF and the other processed LLF groups. Differential components were screened according to the criteria of variable importance in projection (VIP) > 1 , $P < 0.05$, and $|\log_2(\text{fold change})| \geq 1$.

3. Results

3.1. Anti-PMOP pharmacodynamic evaluation of different processed LLF preparations

3.1.1. General status

The experimental animals were divided into eight groups (Sham, OVX, AS, SLLF, QLLF, JLLF, CLLF, YLLF groups). All processed LLF preparations were administered at the same crude-drug equivalent dose to ensure direct comparability among different processing methods. All treatments were given once daily by oral gavage for 12 weeks (Fig. 2A). All LLF intervention groups reduced adiposity versus the OVX control, with JLLF showing the largest numerical change (Fig. 2B). OVX animals also exhibited severe uterine atrophy reflecting estrogen deprivation-induced involution of the reproductive tract. All LLF formulations significantly reversed this atrophy; JLLF restored uterine weight closest to sham levels, indicating the strongest uterotrophic activity (Fig. 2C).

3.1.2. Histopathological analysis of femoral bone tissue

Histopathological analysis of femoral bone tissue using H&E staining showed that sham-operated controls had intact trabecular microarchitecture with minimal marrow fat. In contrast, OVX specimens displayed typical signs of osteoporosis, such as reduced trabecular density, structural gaps, larger marrow spaces, and increased fat cells, confirming successful model establishment (Fig. 2D). Notably, JLLF markedly preserved trabecular integrity and attenuated marrow adiposity, while YLLF conferred comparable, albeit attenuated, protective effects. Quantitative morphometry within standardized ROIs demonstrated substantially diminished trabecular area fraction in OVX, significantly ameliorated by alendronate and JLLF, with concomitant reduction in marrow fat fraction across all treatment arms (**Figure S2 and Fig. 2E**). JLLF exhibited paramount osteoanabolic efficacy, augmenting trabecular number and connectivity while minimising adipocyte accrual. Masson trichrome and TRAP staining substantiated these findings (Fig. 2F). OVX animals displayed attenuated collagen deposition, evidenced by diminished blue intensity, and elevated TRAP-positive osteoclasts, indicative of compromised matrix integrity and heightened skeletal fragility. JLLF increased collagen content and reduced osteoclast-related staining compared with the OVX group.

3.1.3. Femoral microarchitecture

Micro-CT morphometry revealed profound trabecular deterioration in OVX animals, manifested by marked reductions in BV/TV, Tb.Th, Tb.N, and Conn.Dn, concomitant with elevated BS/BV, Tb.Sp, Tb.Pf, and SMI, indicative of rod-like transformation of plate-like architecture and successful model induction (Fig. 3A). Following 12-week intervention, JLLF demonstrated comprehensive restoration of all eight parameters, with efficacy approximating that of alendronate. YLLF significantly ameliorated seven indices, while QLLF improved five; raw and vinegar-steamed products exhibited comparatively modest effects. Notably, only JLLF significantly reversed the plate-to-rod transition as reflected by SMI. Three-dimensional reconstructions corroborated these quantitative findings: OVX specimens exhibited trabecular rarefaction and discontinuity, whereas JLLF and YLLF preserved dense, well-connected bony networks with diminished intertrabecular spacing (Fig. 3B). Densitometric analysis further demonstrated that JLLF elevated trabecular and cortical BMD by 5.23% and 5.58%, respectively ($P < 0.01$ vs OVX), showed the largest numerical change among the tested preparations (Fig. 3C). Cortical morphometry revealed that raw, JLLF, CLLF, and YLLF significantly increased Tt.Ar ($P < 0.05$), with JLLF and YLLF additionally augmenting Ct.Th (Fig. 3D). Collectively, these data substantiate that wine-steaming confers the broadest set of improvements among the tested preparations at the selected dose among LLF processing methods, effecting comprehensive reversal of trabecular degeneration, enhancement of bone mineral density, and amelioration of cortical geometry in estrogen-deficient osteoporosis.

3.1.4. Bone biomechanics

Tibial structural integrity was assessed by three-point bending to failure, with synchronous acquisition of load-deflection data. OVX animals exhibited profound biomechanical compromise: attenuated maximum

load, fracture stress, fracture strain, elastic modulus, fracture deflection, bending energy, and stiffness coefficient, concomitant with diminished curve gradients, indicative of impaired structural integrity, reduced toughness, and heightened fragility (Fig. 4A). while raw, steamed, vinegar-steamed, and salt-steamed products elicited partial restoration of these parameters, JLLF effected comprehensive biomechanical rehabilitation, restoring all seven indices with load-deflection profiles approximating sham-operated levels and fracture deflection within physiological limits, demonstrating superior efficacy in reinstating bone strength and structural resilience in estrogen-deficient osteoporosis.

3.1.5. Bone turnover

Ovariectomy-induced estrogen deprivation precipitated a catabolic bone turnover state, characterized by suppressed osteoblastic activity as evidenced by diminished serum ALP, BALP, and OCN (vs Sham, Fig. 4B). JLLF and YLLF significantly restored these formation markers, suggesting attenuation of high-turnover bone loss. Urinary DPD, a specific resorption marker, was markedly elevated in OVX and significantly attenuated across all treatment groups, with JLLF and YLLF exhibiting the largest numerical change, indicative of suppressed osteoclastic activity and restored turnover equilibrium.

3.1.6. Inflammatory cytokines

Compared with the Sham group, OVX animals exhibited elevated IL-17 and TNF- α concomitant with reduced TGF- β and IL-10 (Fig. 4C, 4D). JLLF, YLLF, and SLLF significantly restored TGF- β to sham-operated levels, while all processed products attenuated TNF- α , and all except YLLF reduced IL-17.

3.1.7. Lipid homeostasis

Compared with the Sham group, OVX rats exhibited significantly elevated TC, TG, and LDL-C, whereas HDL-C was markedly reduced (Fig. 4E), consistent with estrogen-deficiency-induced metabolic dysregulation. JLLF elicited comprehensive restoration of lipid homeostasis, attenuating TC (18.7%), TG (22.4%), and LDL-C (25.6%) while augmenting HDL-C (19.8%); this efficacy showed the largest numerical changes among the tested preparations. YLLF and CLLF partially ameliorated lipid profiles; QLLF exerted negligible systemic effects.

3.2. Serum chemical profiling of different processed LLFs

3.2.1. In vivo characterization of LLF components

The serum metabolite profiles following oral administration of five processed LLF preparations in OVX rats are depicted in **Figure S3**. A total of 129 components were annotated, consisting of 55 prototype compounds, which included 12 iridoid glycosides, 10 flavonoids, 6 phenylethanoid glycosides, 6 triterpenes, 3 phenylpropanoids, and 18 miscellaneous constituents, along with 74 phase I/II metabolites (**Tables S1 and S2**). The overall detection rates varied across preparations: SLLF (102), QLLF (116), JLLF (124), CLLF (118), and YLLF (118) (Fig. 5A), with 40, 45, 51, 47, and 46 prototype compounds, respectively (Fig. 5B). Notably, 92 components constituted a core metabolome shared among all

treatment groups. The metabolic transformations were predominantly Phase I reactions (demethylation, hydroxylation, dehydration, and decarboxylation) and Phase II conjugations (glucuronidation and sulfation).

3.2.2. Multivariate analysis of differential LLF metabolites

A targeted LC-MS workflow was used for relative serum chemical profiling of 129 serum constituents across five processed LLF preparations. Representative extracted ion chromatograms acquired in positive ion mode are presented in (Fig. 5C). Following internal standard calibration and peak area normalization to total ion current, unsupervised PCA and supervised PLS-DA were conducted. As illustrated in (Fig. 5D and 5E), both models demonstrated clear intergroup separation. Notably, JLLF and YLLF samples clustered with the highest proximity, indicating substantial similarity in their serum chemical profiles at the sampling time point, whereas SLLF exhibited the most pronounced metabolic divergence from all other preparations. A total of 51 differentially abundant metabolites were identified as potential discriminatory markers based on $VIP > 1.0$, including 14 iridoid glycosides, 6 phenylethanoid glycosides, 6 triterpenes, 3 phenylpropanoids, and 22 structurally diverse constituents (**Table S3**).

3.2.3. Differences in in vivo translocated components between JLLF and other LLF preparations

To systematically analyze the differences in serum translocated components of different processed LLF products in OVX rats, OPLS-DA was performed on samples from other groups. With $|\log_2(FC)| \geq 1$, $VIP > 1$, and T-test $P < 0.05$ as the screening criteria, potential differential components between groups were identified. The differential components obtained from pairwise comparisons of JLLF with the other four processed products (SLLF, QLLF, CLLF, YLLF) are detailed in (**Table S4**). The OPLS-DA model was validated by 200 response permutation tests and supplemented with S-plot visualization (**Figure S4**). The number of differential components between groups was as follows: JLLF vs SLLF (41), JLLF vs QLLF (47), JLLF vs CLLF (31), and JLLF vs YLLF (25). The primary differential substances included prototype components such as salidroside, ligustrosidic acid, loganic acid, tormentic acid, syringin, and their corresponding recovered metabolites.

3.3. Digital dissection of JLLF bioactive components via multi-strategy spectrum-efficacy correlation

3.3.1. Bivariate correlation analysis

Given substantial peak area variability among absorbed LLF constituents and divergent directional correlations between pharmacodynamic indices and therapeutic outcomes, chemical spectral data were subjected to dimensionless standardization, while absolute pharmacodynamic values were transformed into corresponding inhibition or promotion rates to eliminate dimensional disparities and unify effect evaluation directionality. Bivariate correlation models were systematically constructed across six dimensions: bone histopathology, femoral microarchitecture, bone biomechanics, bone turnover,

inflammatory cytokines, and lipid profiles. Significantly correlated components totaled 62, distributed as 14, 22, 25, 10, 25, and 13 for each dimension, respectively (Fig. 6A), involving 22 prototypes including oleanolic acid, specnuezhenide, salidroside, tormentic acid, ursolic acid, secoiridoid glycosides, and ligustrosidic acid, alongside their metabolites (Fig. 6B). These findings indicate associations of multiple serum components, including metabolites, with the measured pharmacodynamic endpoints.

3.3.2. Entropy-weighted grey relational analysis

To precisely screen key serum components significantly associated with composite pharmacodynamic indices, a dual entropy weighting method was employed to assign objective weights to individual indicators and six-dimensional parameters, generating composite efficacy scores (Fig. 6C). Ranked scores were: JLLF (0.9922) > YLLF (0.4900) > CLLF (0.3147) > SLLF (0.3095) > QLLF (0.1486), corroborating JLLF's superior anti-osteoporotic efficacy. Grey relational analysis (GRA) subsequently associated LLF metabolic profiles with composite efficacy indices. Fifty-seven components exhibited correlation coefficients > 0.8, predominantly comprising 19 prototypes (lusianin, oleanolic acid, specnuezhenide, tormentic acid, luteolin-7-O-glucoside, ursolic acid) and 26 metabolites, including ligustrosidic acid, tormentic acid, and syringin.

3.3.3. Multi-strategy integration for candidate component prioritization

To pinpoint JLLF-specific advantageous constituents, an integrated “differential component-efficacy” screening strategy was established, requiring that candidate components simultaneously satisfied: (1) significantly higher peak area in JLLF versus other preparations with multivariate statistical analysis; (2) bivariate and grey correlation coefficients > 0.8 with composite efficacy indices. Twenty-five JLLF-characteristic bioactive components were thereby prioritized as candidates, encompassing 16 prototypes, including (hydroxytyrosol, salidroside, protocathechuic acid) and 9 metabolites, including loganin, arbutin, ligustrosidic acid (Fig. 6D). Correlation heatmap analysis (Fig. 6E) demonstrated significant positive correlations between these 25 components and all six pharmacodynamic dimensions, substantiating their role as the candidate constituents associated with the observed effects underlying the wine-steaming-enhanced efficacy of JLLF.

3.4. Spatial distribution characteristics of JLLF-characteristic differential constituents in renal tissues

DESI-MSI ion images of 14 representative JLLF-characteristic bioactive constituents in sagittal rat kidney sections detected in $[M + H]^+$ or $[M - H]^-$ ionization mode, with signal intensity color-coded from blue for low abundance to red/orange for high abundance, revealing four distinct anatomically heterogeneous distribution patterns closely associated with molecular size and physicochemical properties (Fig. 7). Eight constituents including specnuezhenide, oleoside, ligustrosidic acid, syringin, lusianin, cynaroside, loganic acid and tormentic acid show strictly cortex-restricted enrichment, forming a continuous intense annular band in the peripheral renal cortex with negligible signals in the medulla and pelvis; salidroside

and tyrosol exhibit a biphasic cortex-pelvis distribution with high abundance in both the peripheral cortex and peripelvic tissue and the lowest signals in the intervening medulla; arbutin and hydroxytyrosol display globally weak signals across the entire renal parenchyma, indicating limited tissue retention of these highly polar small molecules; loganin and ursolic acid present a diffuse distribution with mild cortical preference, showing broader tissue penetration with appreciable abundance throughout the medulla. These spatially diversified distribution profiles provide a tissue-level material basis for JLLF to exert region-specific regulatory effects on renal metabolism.

3.5 Spatial metabolomics characterizes regional metabolic alterations in renal tissues.

Spatial metabolomic imaging revealed pronounced spatial heterogeneity in the distribution of tryptophan-related metabolites across different renal anatomical regions. L-5-Hydroxytryptophan was predominantly localized in the renal pelvis and renal medulla, and the renal pelvis, renal medulla was selected as the ROI for semi-quantitative analysis (Fig. 8). In contrast, 5-Hydroxyindole-3-acetic acid showed a relatively broad distribution throughout the whole kidney, while indole-3-ethanol and indole-3-acetamide were mainly distributed across the renal medulla and renal cortex. Indoleacetate exhibited a more restricted distribution, predominantly localized in the renal medulla, highlighting the marked spatial heterogeneity of tryptophan metabolites within the kidney. Compared with the Control group, the Model group showed apparent alterations in the spatial signal intensities of these metabolites. L-5-Hydroxytryptophan, 5-Hydroxyindole-3-acetic acid, and indole-3-ethanol showed increased signals, whereas indoleacetate and indole-3-acetamide showed decreased signals, indicating disrupted tryptophan metabolic homeostasis within specific renal regions under pathological conditions. ROI-based semi-quantitative analysis further demonstrated distinct regulatory effects of different processing methods of LLF. For L-5-hydroxytryptophan, the elevated Model-group signal was reduced following treatment, with wine-processed JLLF showing the strongest restoration toward the Control pattern. Similar trends were observed for 5-hydroxy-indoleacetate and indole-3-ethanol, with JLLF producing the most pronounced reduction in pathological signals, followed by salt-processed YLLF. Conversely, indoleacetate, mainly localized in the renal medulla, was reduced in the Model group and partially restored following intervention, particularly after wine and salt processing. The reduced signal of indole-3-acetamide, distributed mainly in the renal medulla and cortex, was also partially restored, with the most apparent recovery observed in the wine-processed group.

Furthermore, metabolic pathways were also enriched in the Secondary bile acid biosynthesis (Fig. 9). Consistent with the alterations observed in tryptophan metabolism, spatial metabolomic analysis revealed pronounced regional heterogeneity in secondary bile acid-related metabolites. Lithocholic acid was predominantly localized in the renal medulla, whereas deoxycholic acid and 7-ketolithocholic acid were mainly distributed in the renal cortex. Compared with the Control group, the Model group showed decreased medullary lithocholic acid signals but increased cortical signals of deoxycholic acid and 7-ketolithocholic acid, indicating disrupted renal secondary bile acid metabolism. ROI-based semi-

quantitative analysis further demonstrated distinct regulatory effects of different processing methods. The reduced lithocholic acid signal was most prominently restored by JLLF followed by YLLF, while the elevated signals of deoxycholic acid and 7-ketolithocholic acid were correspondingly reduced, particularly following wine and salt processing. Overall, wine processing showed the strongest regulatory effect, followed by salt processing, whereas raw and steamed preparations showed moderate effects and vinegar processing exhibited the weakest overall regulation.

4. Discussion

Ovariectomy-induced estrogen depletion triggers systemic metabolic perturbations; the observed adiposity, uterine atrophy, and trabecular deterioration in OVX rats faithfully recapitulate the high-turnover bone loss phenotype of PMOP^{15,16,33}. JLLF exhibited the most comprehensive pharmacological effects among the five preparations at the tested dose, improving bone biomechanical properties, modulating bone turnover markers by increasing osteoblastic indicators (ALP, BALP, OCN) and reducing the resorption marker DPD, attenuating OVX-associated inflammatory alterations, and improving dyslipidemia and marrow adiposity. Micro-CT and histomorphometric analyses further supported the enhanced protective effects of JLLF on trabecular connectivity and bone mineral density compared with other processed LLF preparations, with the overall pharmacological performance ranking as JLLF > YLLF > CLLF > SLLF > QLLF under the present experimental conditions. This enhanced pharmacological profile may be associated with wine-steaming-induced changes in chemical composition and transformation of specific constituents, although further pharmacokinetic studies are required to confirm changes in systemic bioavailability^{11,12}. To explore the chemical basis associated with JLLF-related pharmacological effects, a three-tiered spectrum–efficacy correlation framework was established, comprising bivariate correlation, entropy-weighted GRA, and multi-strategy integrated validation. This integrative design reduces the limitations of single-method approaches in evaluating low-abundance constituents and improves the robustness and reproducibility of candidate constituent prioritization^{33,34}. Bivariate correlation across six pharmacodynamic domains identified 62 significantly associated constituents. Entropy-weighted GRA further generated a composite efficacy score, ranking JLLF (0.9922) highest among the tested preparations and evaluating the associations between constituent profiles and overall pharmacodynamic performance, supporting the potential multi-component characteristics of processed LLF³⁶.

An integrated “differential constituent-efficacy” screening strategy was further developed, requiring candidates to simultaneously exhibit significantly higher abundance in JLLF (VIP > 1, P < 0.05 in OPLS-DA) and correlation coefficients > 0.8 in both bivariate and GRA analyses. Triple filtering yielded 25 JLLF-characteristic candidate constituents (16 prototypes, 9 phase I/II metabolites). This strategy eliminates both non-differential efficacy-associated components and chemically differential but pharmacologically irrelevant false positives, prioritizing candidates specifically linked to wine-steaming-enhanced efficacy. Notably, *in vivo* metabolites including loganin, arbutin, and syringin showed stronger correlations with composite efficacy than some prototype compounds, suggesting that biotransformation may contribute

to the pharmacological profile of JLLF^{11,12}. Functional annotation suggested that the 25 candidate constituents are associated with multiple pharmacological pathways related to bone biomechanics, turnover, inflammation, lipid metabolism, and microarchitecture. For instance, loganin, ursolic acid and syringin modulate osteoblast/osteoclast coupling and bone mechanical integrity^{37,38}; luteolin-7-O-glucoside and salidroside attenuate oxidative stress and inflammatory bone damage via HIF-1 α signaling^{39,40}. These findings suggest that the pharmacological effects of JLLF may involve multiple constituents and pathways rather than a single dominant compound.

To elucidate tissue-level mechanisms, DESI-MSI spatial metabolomics was applied to visualize JLLF constituent distribution and metabolic regulation in renal tissues. JLLF-characteristic constituents exhibited pronounced anatomical heterogeneity: small-molecule phenols including hydroxytyrosol, salidroside were enriched in the renal medulla and pelvis, whereas triterpenes and iridoid glycosides such as oleanolic acid, specnuezhenide localized predominantly to the renal cortex, providing a structural basis for region-specific metabolic regulation. OVX induced marked regional dysregulation of renal tryptophan metabolism and secondary bile acid biosynthesis, consistent with reported estrogen deficiency-induced renal metabolic disruption linked to the kidney-bone axis⁴¹. JLLF most effectively reversed these regional metabolic abnormalities, restoring the spatial homeostasis of key metabolites (L-5-hydroxytryptophan, lithocholic acid, deoxycholic acid) to near-sham levels. Modulation of renal tryptophan metabolism may ameliorate systemic inflammation via the kynurenine pathway, while regulation of secondary bile acid metabolism may activate FXR signaling to coordinate bone and lipid homeostasis^{42,43}. Together, these findings validate the TCM theory of “the kidney governing bones” from a spatial metabolomics perspective. Further analysis confirmed that OVX-induced renal metabolic disturbances involve not only altered metabolite abundance but also pronounced regional specificity for both tryptophan and secondary bile acid pathways, extending prior observations of systemic bile acid dysregulation in estrogen deficiency to the tissue microenvironment^{44,45}. The graded regulatory effects across processed LLF preparations were strongest for wine-steaming, followed by salt-steaming, indicating that processing methods directionally modulate renal metabolic homeostasis, which may contribute to their differential osteoprotective efficacy via the kidney-bone axis .

In conclusion, this study systematically compared the pharmacological profiles of five processed LLF preparations in an OVX rat model of PMOP and demonstrated that JLLF exhibited the most comprehensive effects among the tested preparations using an integrated strategy combining multidimensional pharmacodynamics, serum pharmacochemistry, spectrum–efficacy correlation, and DESI-MSI spatial metabolomics. An integrated “pharmacodynamics–serum chemistry–spatial metabolomics” strategy was applied to evaluate TCM processing effects and provide a comprehensive approach for investigating candidate constituents and associated metabolic alterations. This analytical framework is transferable to mechanistic studies of other processed TCM materials. Wine-steaming selectively increases the serum abundance of characteristic constituents and reshapes their regional distribution profiles in renal tissues. By contrast, vinegar-steamed LLF exhibits only moderate lipid-lowering activity with negligible osteoprotective efficacy, reflecting a processing-specific “therapeutic

target bias” that supports disease-oriented selection of processed products. The identified efficacy-associated constituent panel provides objective quality markers for the transition from empirical processing to data-driven, efficacy-based standardization of LLF preparations. Notably, JLLF effectively reverses ovariectomy-induced regional dysregulation of renal tryptophan metabolism and secondary bile acid biosynthesis, restoring renal spatial metabolic homeostasis. These findings indicate that JLLF exerts systemic anti-PMOP effects by concomitantly modulating inflammatory-immune profiles, lipid homeostasis, and renal regional metabolism, providing mechanistic evidence supporting the holistic TCM principle of “the kidney governing bones and marrow”. Several limitations remain. First, only one dose level was tested per preparation, precluding characterization of dose-response relationships and preparation-specific optimal doses. Second, tissue pharmacokinetic evidence of JLLF constituents in bone, the direct target organ, is still lacking. Third, the identified characteristic components and their efficacy associations require independent replication in larger cohorts. Fourth, the causal link between renal metabolic remodeling and improved bone phenotype remains to be functionally verified. Future work should establish a quantitative “component-tissue metabolism-phenotype” causal model, optimize wine-steaming parameters via response surface methodology, and elucidate the involvement of estrogen receptors in JLLF-mediated osteoprotection.

5. Conclusions

This study utilized a comprehensive multidimensional integrative approach, incorporating pharmacodynamic evaluation, serum pharmacochemistry, spectral-effect correlation, and DESI-MSI spatial metabolomics, to systematically compare the interventional efficacies and candidate mechanistic associations of various processed LLF preparations in OVX rats modeling PMOP. The pharmacodynamic findings showed that JLLF produced the broadest improvements among the five preparations at the tested dose for LLF in PMOP intervention. By employing a stepwise convergent analytical framework that integrates bivariate correlation, entropy-weighted grey relational analysis, differential constituent screening, and integrated pharmacodynamic validation, the study combined complementary analytical approaches and mitigated limitations associated with single-strategy approaches. In this study, we prioritized 25 JLLF-characteristic candidate constituents, including 16 parent compounds and 9 in vivo metabolites, which were associated with the enhanced pharmacological effects observed after wine-steaming processing. These candidate constituents showed associations with multiple pharmacodynamic domains, including bone biomechanics, metabolism, inflammation, lipid handling, and microarchitecture. Spatial metabolomics analysis further revealed that JLLF characteristic components exhibited distinct regional localization in renal tissues, and JLLF treatment was associated with the modulation of OVX-induced alterations in renal tryptophan metabolism and secondary bile acid biosynthesis, providing insights into renal metabolic changes related to JLLF intervention. The findings provide experimental support for further investigation of processed LLF products, including validation of candidate constituents, metabolic pathways, and potential translational relevance.

Abbreviations

ALP	Alkaline phosphatase
AS	Alendronate sodium
BALP	Bone-specific alkaline phosphatase
BMD	Bone mineral density
CLLF	Vinegar-steamed LLF
DPD	Deoxypyridinoline
GO	Gene Ontology
GRA	Grey relational analysis
HDL-C	High-density lipoprotein cholesterol
IL-10	Interleukin-10
IL-17	Interleukin-17
JLLF	Wine-steamed LLF
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS	Liquid chromatography-mass spectrometry
LDL-C	Low-density lipoprotein cholesterol
LLF	<i>Ligustri Lucidi Fructus</i>
OCN	Osteocalcin
OPLS-DA	Orthogonal partial least squares discriminant analysis
OVX	Ovariectomized
PCA	Principal component analysis
PLS-DA	Partial least squares discriminant analysis
PLSR	Partial least squares regression
PMOP	Postmenopausal osteoporosis
PPI	Protein-protein interaction
QLLF	Steamed LLF
RMSD	Root-mean-square deviation
RMSF	Root-mean-square fluctuation
SLLF	Raw LLF
TC	Total cholesterol

TCM	Traditional Chinese medicine
TGF- β	Transforming growth factor beta
TG	Triglycerides
TNF- α	Tumor necrosis factor alpha
YLLF	Salt-steamed LLF

Declarations

Data availability statement

The original contributions presented in the study are included in the article and Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the Experimental Animal Ethics Committee of Beijing University of Chinese Medicine (protocol code BUCM-2024080201-3070). The study was conducted in accordance with local legislation and institutional requirements.

Author contributions

BZ: Conceptualization, Formal analysis, Investigation, Data curation, Writing - original draft. **SL:** Formal analysis, Data curation, Visualization. **RW:** Formal analysis, Data curation. **TQ:** Visualization. **TK:** Writing - review and editing, Visualization. **XL:** Writing - review and editing. **XH:** Supervision, Project administration, Funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

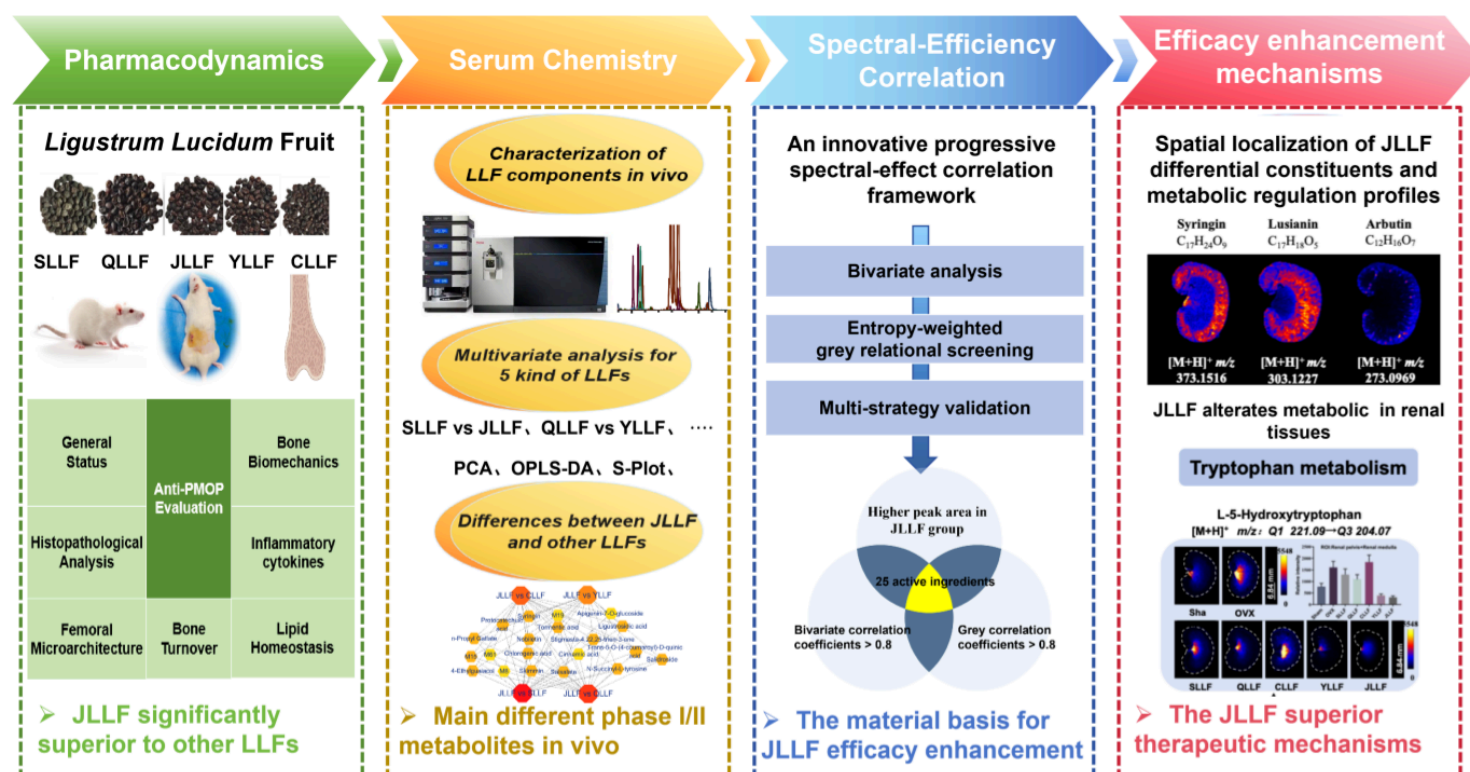


Figure 1

Overview of the integrated research strategy. A multidimensional workflow combining pharmacodynamic evaluation, serum pharmacochemistry, spectrum–efficacy correlation analysis, and DESI-MSI-based spatial metabolomics was established to investigate the bioactive constituents and tissue-level metabolic mechanisms underlying the anti-postmenopausal osteoporosis effects of wine-steamed *Ligustri Lucidi Fructus* (JLLF).

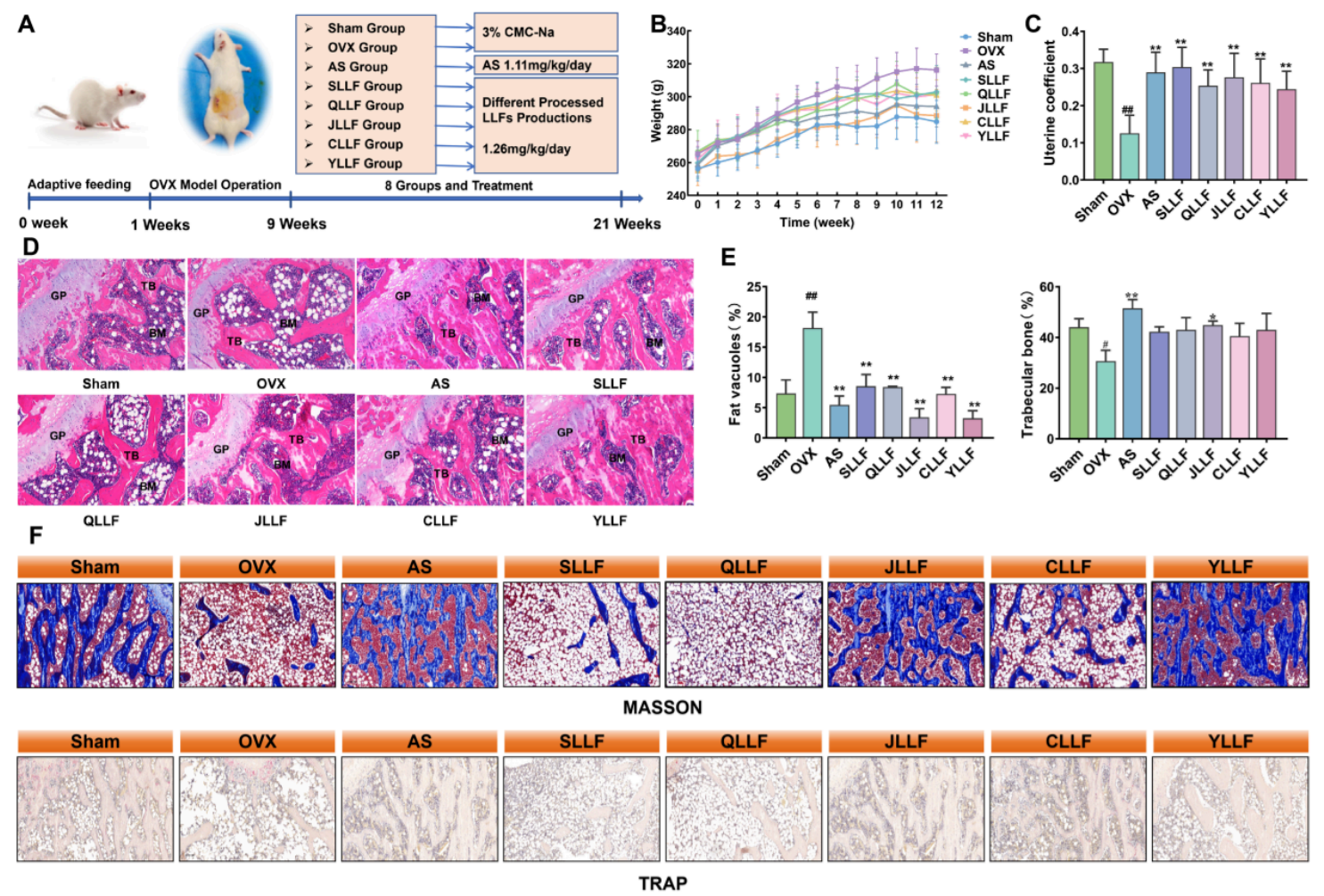


Figure 2

Effects of processed LLFs on physiology and femoral histomorphology in OVX rats (n=12). (A) Timeline of OVX surgery and 12-week treatment. (B) Body weight changes during LLF intervention. (C) Uterine index at necropsy. (D) H&E-stained distal femoral sections (×200), showing growth plate (GP), trabecular bone (TB), and bone marrow (BM). (E) Trabecular bone area fraction and marrow adiposity in standardized ROIs. (F) Masson trichrome and TRAP staining of distal femoral metaphyses.

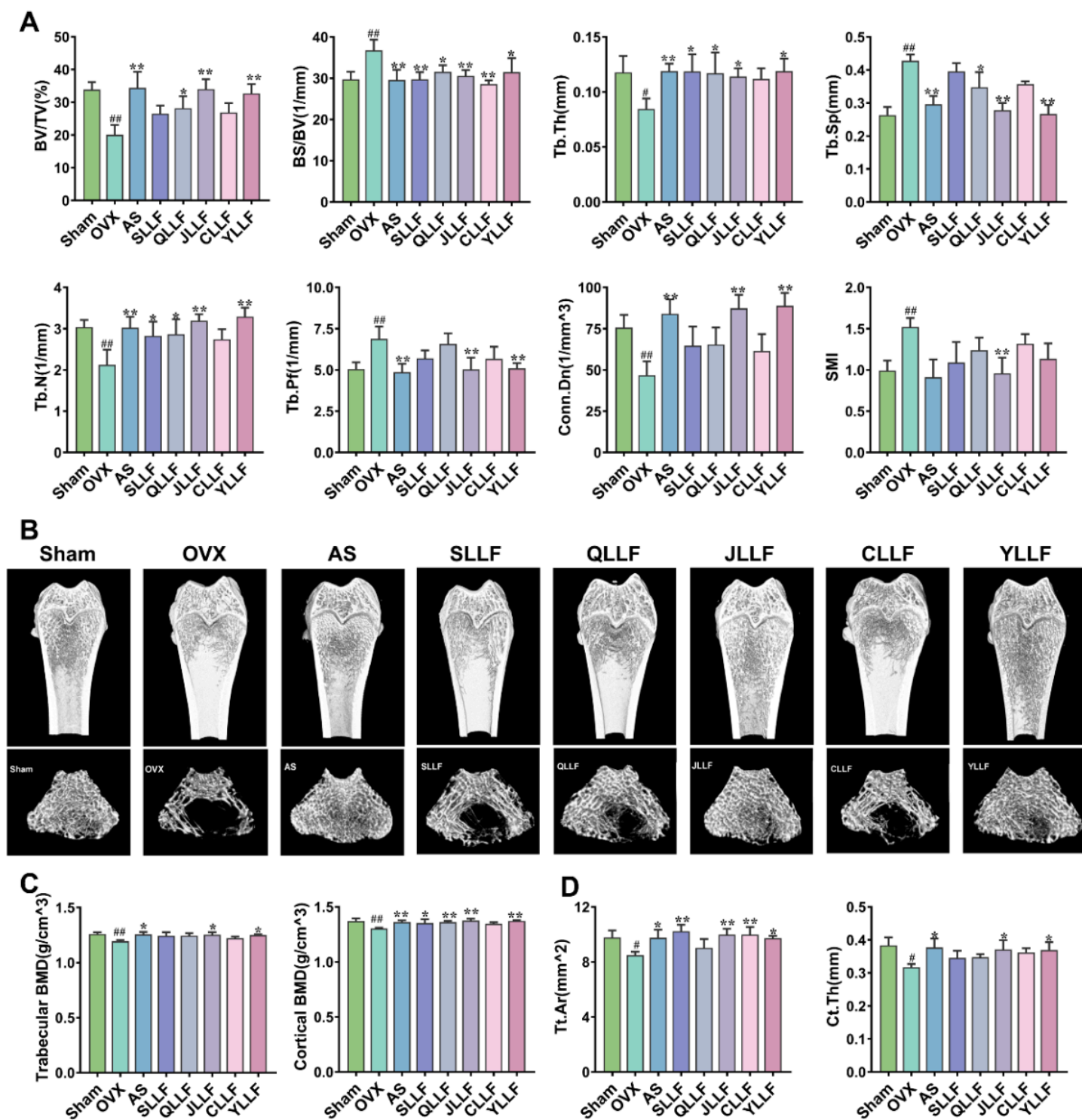


Figure 3

Effects of processed LLFs on femoral microstructure and bone mass in OVX rats. (A) Trabecular parameters: BV/TV, BS/BV, Tb.Th, Tb.Sp, Tb.N, Tb.Pf, Conn.Dn, SMI. (B) 3D micro-CT reconstructions of distal femoral metaphyses. (C) Trabecular and cortical BMD. (D) Cortical parameters: Tt.Ar and Ct.Th.

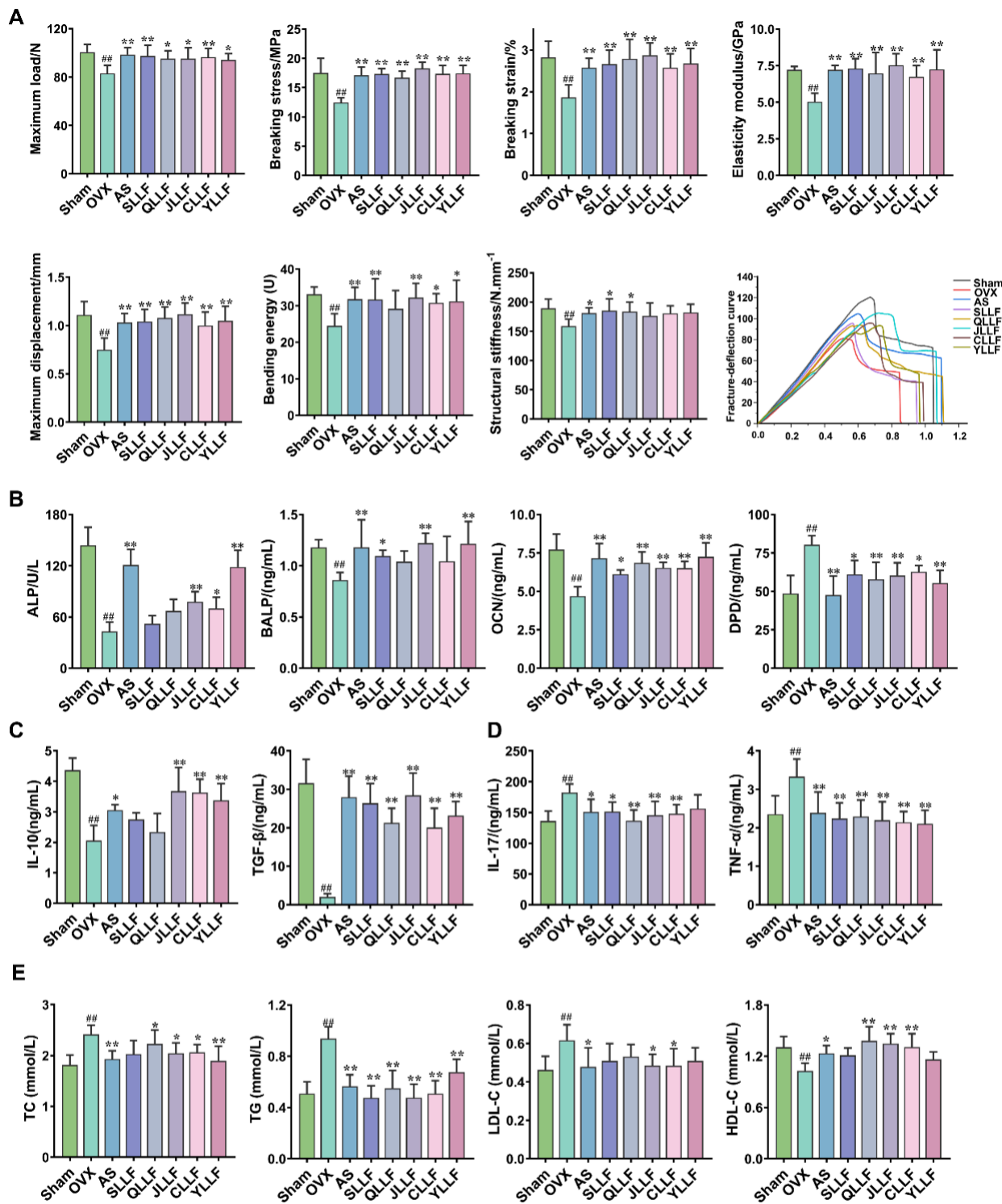


Figure 4

Effects of processed LLFs on bone strength, turnover, inflammation, and lipids in OVX rats. (A) Tibial biomechanics: maximum load, breaking stress, breaking strain, elasticity modulus, maximum displacement, bending energy, structural stiffness and fracture-deflection curves. (B) Bone turnover markers: serum ALP, BALP, OCN; urinary DPD. (C) Anti-inflammatory mediators: IL-10, TGF-β. (D) Pro-inflammatory cytokines: IL-17, TNF-α. (E) Serum lipids: TC, TG, LDL-C, HDL-C.

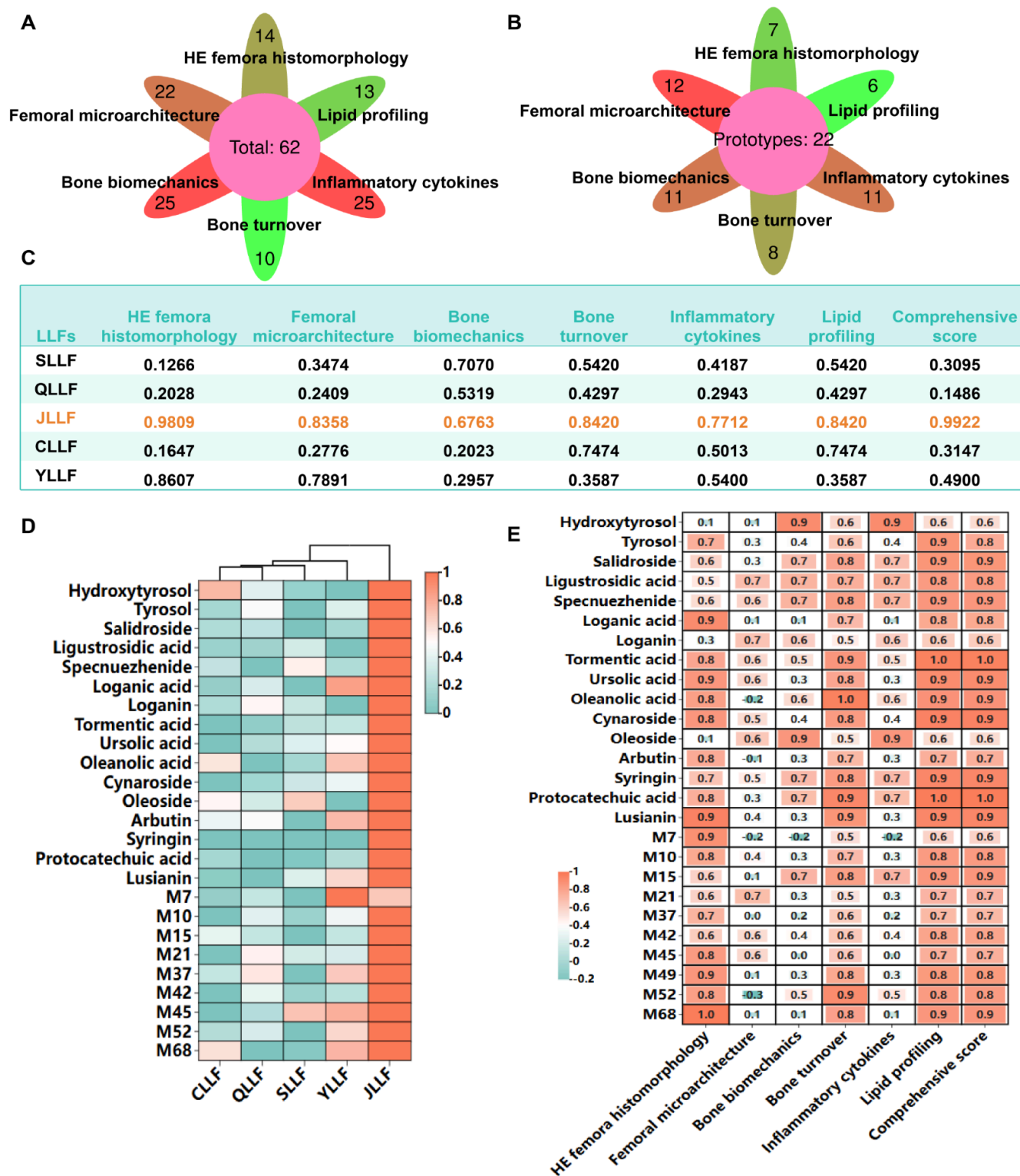


Figure 6

Differentiation analysis of processed LLFs on anti-PMOP via multi-strategy spectroscopic-pharmacodynamic correlation. (A) Bivariate analysis for identifying in vivo components linked to six PMOP pharmacodynamic indicators. (B) Screening of LLF components related to six PMOP indicators. (C) Entropy weight-based scoring of anti-PMOP effects of processed products. (D) Heatmap showing in

vivo content of core pharmacodynamic components in processed products. (E) Heatmap depicting correlation between core components and multi-dimensional anti-PMOP indicators.

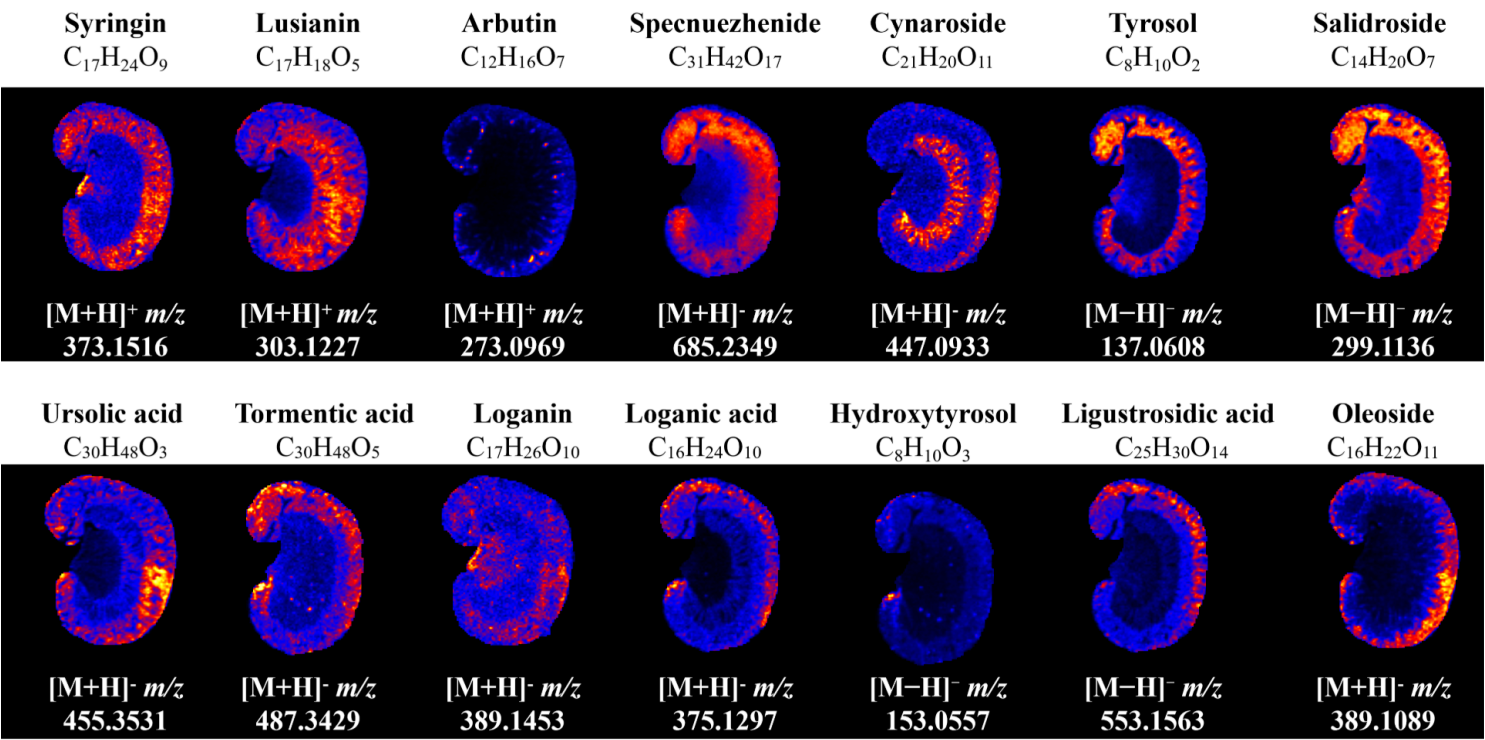


Figure 7

Spatial distribution of JLLF-characteristic differential constituents in rat renal tissues analyzed by DESI-MSI.

Tryptophan metabolism

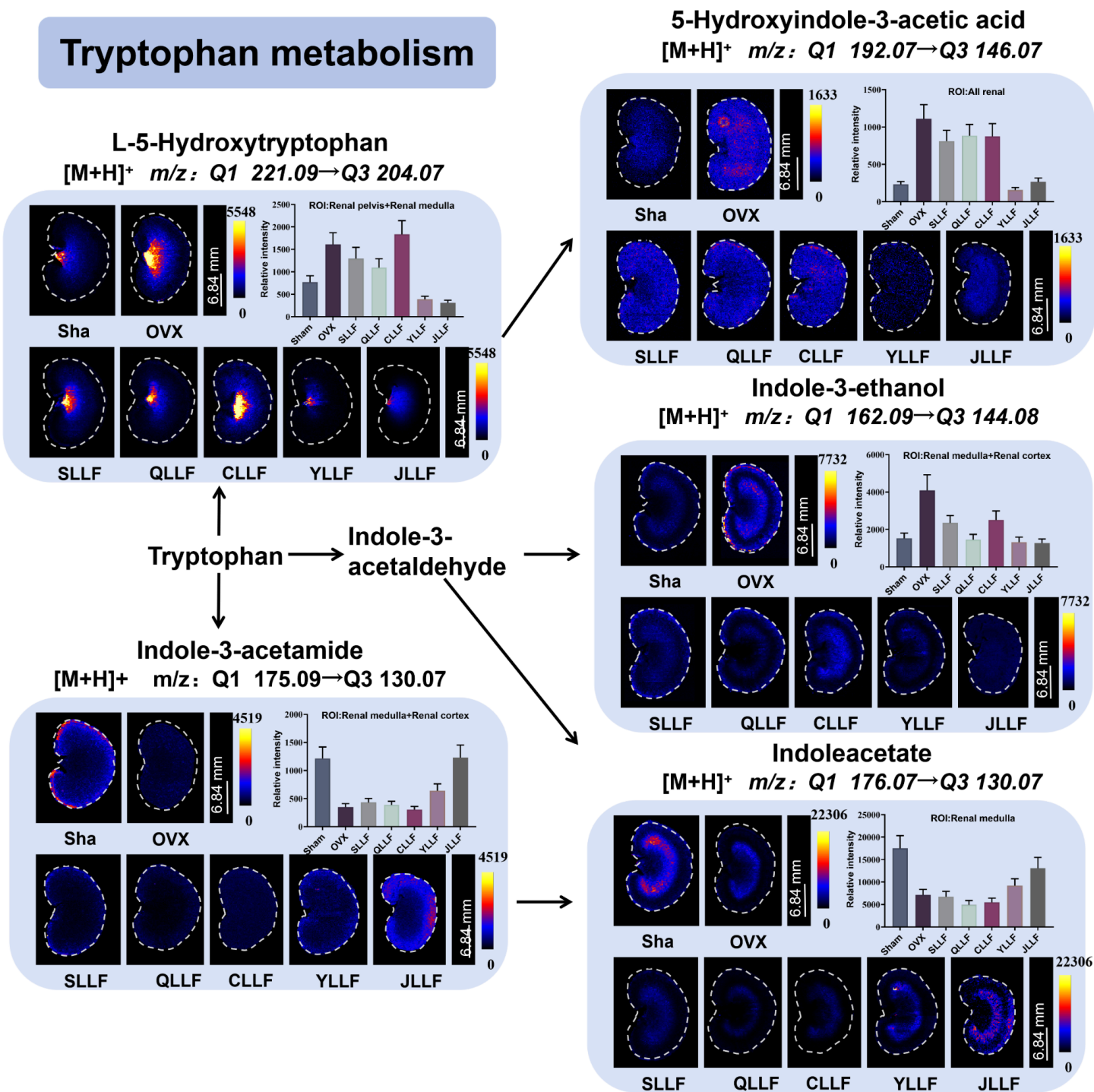


Figure 8

Integrated cascade diagram of the Tryptophan metabolism pathway and mass spectrometry imaging.

Secondary bile acid biosynthesis

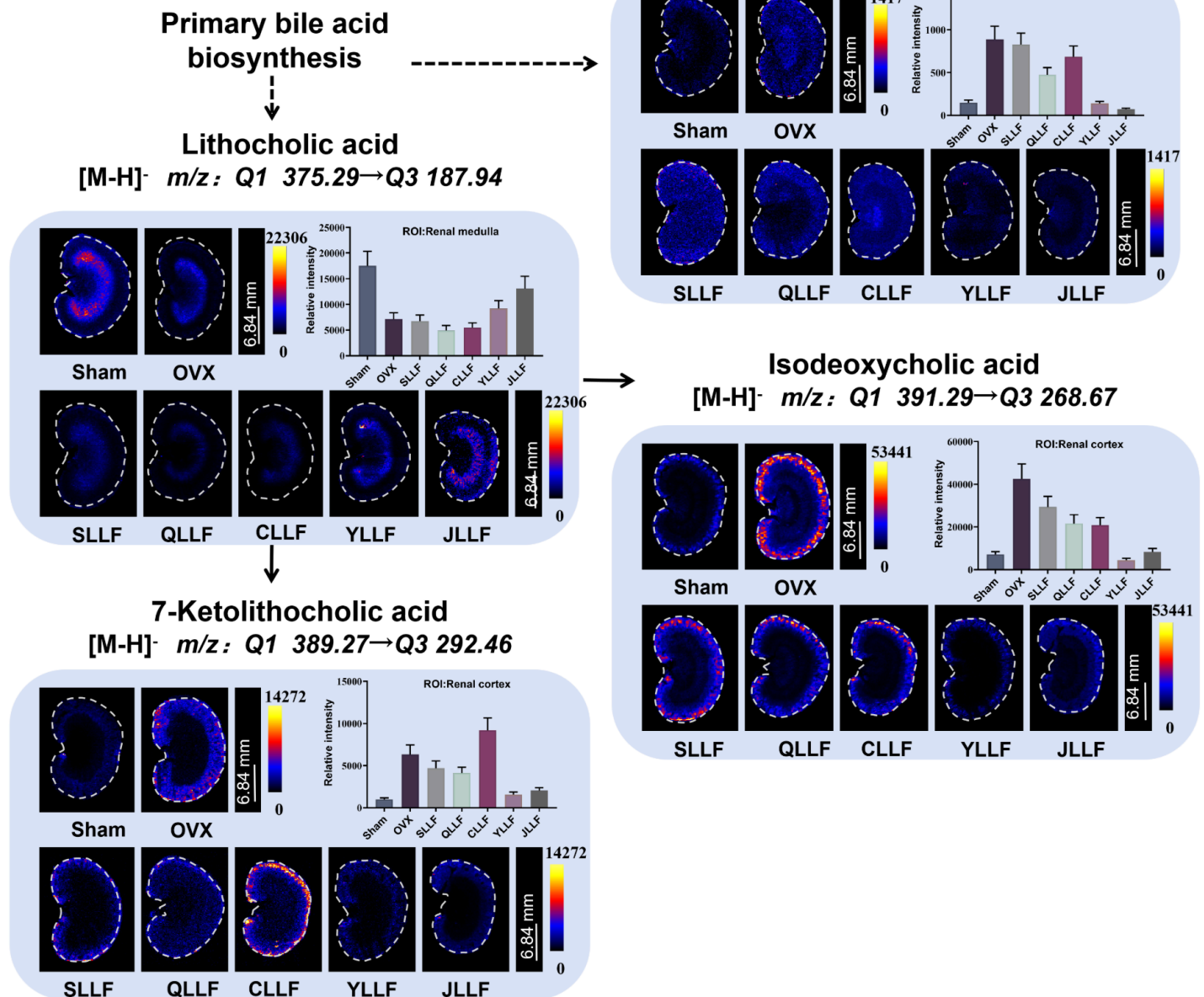


Figure 9

Integrated cascade diagram of the Secondary bile acid biosynthesis pathway and mass spectrometry imaging.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterials.docx](#)