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EVALUATION OF BIOACTIVE COMPOUNDS, ANTIOXIDANT ACTIVITY, ANTI-ACNE AND ALNAGESIC ACTIVITY OF CHINESE MOTHERWORT (*LEONURUS JAPONICUS*) DIFFERENT PART EXTRACTS

Nakornsub Lawcharoen, L. E. Kanyaphat, Sutthikiat Udommethaporn and Alinthipat Kanchaburin*

School of Integrative, Mae Fah Luang University, 333 Moo1 Thasud, Mueang, Chiangrai 57100, Thailand.

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Correspondence to Author: Alinthipat Kanchaburin

School of Integrative,
Mae Fah Luang University,
333 Moo1 Thasud, Mueang,
Chiangrai 57100, Thailand.

E-mail: alinthipat.kan@mfu.ac.th

ABSTRACT: *Leonurus japonicus* Houtt. (Lamiaceae), commonly known as Chinese motherwort or Yi-Mu-Cao, has a longstanding history in traditional Asian medicine. This study comparatively evaluated the phytochemical constituents, antioxidant capacity, anti-acne properties, and *in-vitro* anti-inflammatory activities of hot-water extracts obtained from the aerial parts and roots of *L. japonicus* cultivated in Northern Thailand. Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophotometrically, while antioxidant capacities were evaluated using DPPH and FRAP assays. Antibacterial activity against acne-associated pathogens (*Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) was assessed by broth microdilution to determine minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations. *In-vitro* anti-inflammatory activity was quantified by measuring prostaglandin E₂ (PGE₂) inhibition in LPS-stimulated RAW 264.7 macrophages via enzyme-linked immunosorbent assay (ELISA). The aerial part extract contained significantly higher TPC (26.35 ± 0.11 mg GAE/g) and TFC (17.89 ± 0.19 mg QE/g) than the root extract (7.40 ± 0.37 mg GAE/g and 5.77 ± 0.14 mg QE/g, respectively; $p < 0.001$). Correspondingly, aerial extracts exhibited superior DPPH (97.14 ± 0.15 mg TE/g) and FRAP (90.20 ± 0.35 mg TE/g) values. Aerial extracts demonstrated moderate antibacterial activity, inhibiting *C. acnes* at an MIC of 625.0 µg/mL, and *S. aureus* and *S. epidermidis* at 1250.0 µg/mL. Both extracts produced a concentration-dependent suppression of PGE₂ production (0.25–1.50 mg/mL), with the aerial extract achieving 75.8% inhibition at 1.50 mg/mL. These results indicate that aqueous extracts of *L. japonicus* aerial parts possess noteworthy antioxidant, antibacterial, and PGE₂-suppressing properties *in-vitro*, corroborating the traditional preference for aerial structures in herbal preparations.

INTRODUCTION: Species of the genus *Leonurus* (family Lamiaceae) have been utilized extensively in traditional phytotherapy across Asia, Europe, and the Americas¹.

Within this genus, *Leonurus japonicus* Houtt. (syn. *Leonurus heterophyllus* Sweet), vernacularly known as Chinese motherwort or Yi-Mu-Cao, represents an official botanical drug documented in the Pharmacopoeia of the People's Republic of China².

Traditionally, the dried aerial parts collected during summer inflorescence are prescribed to invigorate blood circulation, regulate menstruation, induce diuresis, and resolve edema, whereas the mature fruits, termed Chong-Wei-Zi, are utilized

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separately for ophthalmological and gynecological indications^{1,2}.

Phytochemical and pharmacological investigations on *L.japonicus* have elucidated diverse secondary metabolites, notably alkaloids (such as leonurine and stachydrine), labdane diterpenoids, iridoid glycosides, sterols, and polyphenols^{3, 4}. Modern pharmacological investigations have corroborated that crude extracts and isolated constituents from *L.japonicus* exert broad biological actions, including cardioprotective, anti-inflammatory, antioxidant, neuroprotective, and antimicrobial effects^{3,5}.

While commercial and clinical applications in East Asia predominantly employ aerial biomass extracted with hydroalcoholic mixtures, traditional ethnopharmacological preparations typically involve aqueous decoctions (tang ji). Moreover, comparative studies examining whether anatomical parts (aerial biomass versus underground root structures) differ systematically in secondary metabolite yield and downstream bioactivities specifically in samples acclimatized and cultivated in sub-tropical Southeast Asian environments like Northern Thailand remain scarce.

In traditional medical frameworks, botanical remedies applied for systemic inflammatory syndromes are frequently utilized for concurrent dermatological and gynecological complaints^{2, 3}. Acne vulgaris represents a chronic inflammatory dermatosis of the pilosebaceous unit, primarily driven by hyperkeratinization, excessive seborrhea, and follicular colonization by opportunistic bacteria including *Cutibacterium acnes* (formerly *Propionibacterium acnes*), *Staphylococcus epidermidis*, and *Staphylococcus aureus*^{6, 7}. Colonization triggers the release of pro-inflammatory cytokines and reactive oxygen species (ROS), exacerbating tissue damage⁷. Concurrently, pro-inflammatory lipid mediators, particularly prostaglandin E₂ (PGE₂), generated via the cyclooxygenase-2 (COX-2) enzymatic pathway, play pivotal roles in inflammatory hyperalgesia and smooth muscle spasticity, contributing to common distress conditions such as primary dysmenorrhea^{8, 9}. Exploring botanical extracts capable of quenching free radicals, repressing cutaneous pathogens, and mitigating

cellular PGE₂ generation provides scientific rationale for understanding their multi-target therapeutic potential. Accordingly, the present investigation was designed to systematically evaluate and compare the total phenolic content, total flavonoid content, in vitro antioxidant capacities (DPPH and FRAP), antibacterial activities against acne-inducing pathogens (*C. acnes*, *S. aureus*, and *S. epidermidis*), and in-vitro PGE₂-inhibitory properties of hot-water extracts prepared from the aerial parts and roots of *L. japonicus* cultivated in Northern Thailand.

MATERIALS AND METHODS:

Botanical Collection and Authentication: Fresh specimens of *Leonurus japonicas* Houtt. were collected during the flowering stage (August 2024) from the Botanical Garden of Mae Fah Luang University, Chiang Rai, Thailand (latitude 20°02'48"N, longitude 99°53'45"E, elevation 410 m). Taxonomic identification and botanical authentication were performed by an expert taxonomist at the Medicinal Plant Innovation Center, Mae Fah Luang University. A voucher specimen (No. MFU-NPRC-2024-LJ012) was deposited in the Mae Fah Luang University Herbarium for permanent reference.

The collected plant material was carefully separated into aerial parts (stems, leaves, and inflorescences) and root systems. Samples were thoroughly rinsed with running tap water followed by deionized water to remove extraneous surface debris, cut into uniform segments (1–2 cm), air-dried in a well-ventilated dehydrator at 45°C for 72 h until constant weight, pulverized into a fine powder using a commercial electric grinder (mesh size 60), and maintained in hermetically sealed containers at -20°C protected from light.

Preparation of Hot-Water Decoctions: To reflect the traditional decoction method (tang ji) practiced in Traditional Chinese Medicine, an aqueous extraction procedure was employed. Dried powder from aerial parts or roots (20.0 g) was suspended in 1000 mL of deionized water (solid-to-solvent ratio 1:50, w/v) in a round-bottom flask equipped with a reflux condenser. The suspension was heated at 100°C for 3 h using an electric heating mantle. Following extraction, the slurry was cooled to ambient temperature (25 ± 2°C) and filtered

through Whatman No. 1 filter paper under vacuum. The clarified aqueous filtrates were concentrated using a rotary vacuum evaporator (Büchi, Flawil, Switzerland) at 50°C and subsequently lyophilized in a freeze-dryer (Labconco, Kansas City, MO, USA) at -50°C and 0.05 mbar for 48 h. The resulting dried extract powders were weighed to calculate percentage extraction yields and preserved in desiccated containers at -20°C until experimental analysis

Determination of Bioactive Constituents:

Total Phenolic Content (TPC): The total phenolic content was measured spectrophotometrically using the Folin–Ciocalteu colorimetric method adapted from Mazumder *et al.*¹⁰ with minor modifications. Reconstituted extract solutions (2.0 mg/mL in distilled water; 0.5 mL) were transferred into amber reaction tubes, combined with 2.5 mL of 10% (v/v) aqueous Folin–Ciocalteu reagent, and allowed to equilibrate for 5 min. Thereafter, 2.0 mL of 7.5% (w/v) sodium carbonate (Na₂CO₃) solution was introduced, vortexed vigorously, and incubated in darkness at 25°C for 30 min. The absorbance was determined at 760 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) against a reagent blank. A standard calibration curve was constructed using analytical grade gallic acid prepared across a validated linear concentration range of 10–100 µg/mL ($y = 0.0098x + 0.0215$, $R^2 = 0.9968$). Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract). Analyses were performed in triplicate.

Total Flavonoid Content (TFC): The total flavonoid content was quantified utilizing the aluminum chloride colorimetric method based on the protocol described by Angeloni *et al.*¹¹. An aliquot of the extract solution (0.5 mL) was mixed with 2.0 mL of deionized water and 0.15 mL of 5% (w/v) sodium nitrite (NaNO₂). Following an initial incubation of 6 min at ambient temperature, 0.15 mL of 10% (w/v) aluminum chloride hexahydrate (AlCl₃·6H₂O) was added and allowed to react for 5 min. Subsequently, 2.0 mL of 4% (w/v) sodium hydroxide (NaOH) and 0.7 mL of deionized water were sequentially added to achieve a final volume of 5.5 mL. The reaction mixture was thoroughly agitated and incubated at 25°C for 15 min. Absorbance was recorded at 510 nm against a

blank containing water in place of AlCl₃. Quercetin was utilized as the calibration standard across a linear range of 20–200 µg/mL ($y = 0.0042x + 0.0118$, $R^2 = 0.9975$). Results were expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract). All determinations were carried out in triplicate.

In-vitro Antioxidant Assays:

DPPH Free Radical Scavenging Assay: The scavenging potential against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was evaluated according to Mustafa and Chin¹². Briefly, 1.0 mL of extract solution at diverse concentrations was mixed with 2.0 mL of a freshly prepared methanolic DPPH solution (0.2 mmol/L). The reaction mixture was vortexed thoroughly and maintained in the dark at 25°C for 30 min. The reduction of DPPH absorbance was measured at 517 nm using a spectrophotometer against a pure methanol blank. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was employed as the reference antioxidant across a concentration range of 10–100 µg/mL ($R^2 = 0.9981$). The radical scavenging capacity was calculated and expressed as milligrams of Trolox equivalents per gram of dry extract (mg TE/g extract).

Ferric Reducing Antioxidant Power (FRAP) Assay: The ferric reducing capacity was determined using the FRAP working reagent prepared freshly by combining 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) dissolved in 40 mmol/L HCl, and 20 mmol/L FeCl₃·6H₂O at a volumetric ratio of 10:1:1¹². The reagent was warmed to 37°C prior to use. Extract aliquots (100 µL) were mixed with 3.0 mL of the FRAP working reagent and incubated at 37°C for 30 min under dark conditions. Absorbance was monitored at 593 nm against a reagent blank. Trolox served as the calibration benchmark (25–500 µmol/L, $R^2 = 0.9987$), and ferric reducing antioxidant power was expressed as milligrams of Trolox equivalents per gram of dry extract (mg TE/g extract).

Anti-Acne Antibacterial Assays:

Microbial Strains and Inoculum Preparation: The antibacterial potential was screened against three opportunistic pathogens implicated in acne

pathogenesis: *Cutibacterium acnes* (ATCC 6919), *Staphylococcus aureus* (ATCC 25923), and *Staphylococcus epidermidis* (ATCC 12228). *S. aureus* and *S. epidermidis* were cultivated aerobically on Mueller–Hinton Agar (MHA) and Mueller–Hinton Broth (MHB) at 37°C for 24 h. *C. acnes* was cultured on Brain Heart Infusion (BHI) agar supplemented with 1% (w/v) glucose under strict anaerobic conditions using an anaerobic gas-generating jar system (Oxoid, Basingstoke, UK) at 37°C for 48–72 h. Bacterial suspensions were adjusted in sterile physiological saline (0.85% NaCl) to achieve a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and subsequently diluted to an operational working inoculum of approximately 5.0×10^5 CFU/mL.

Determination of MIC and MBC: The MIC was determined using the broth microdilution technique in sterile 96-well polystyrene microtiter plates in accordance with Clinical and Laboratory Standards Institute (CLSI M07-A10 and M11-A8) guidelines^{13, 14}. Stock solutions of lyophilized extracts were prepared in sterile broth (10.0 mg/mL) and filtered through 0.22 µm syringe filters. Two-fold serial dilutions were performed across the microplate wells to yield final concentrations ranging from 78.13 to 5000.0 µg/mL. Each well was inoculated with 100 µL of the standardized bacterial suspension, bringing the final well volume to 200 µL. Negative control wells (culture broth only) and positive growth control wells (inoculated broth without extract) were included on each plate. Microplates containing *Staphylococcus* species were incubated aerobically at 37°C for 24 h, whereas *C. acnes* microplates were maintained anaerobically at 37°C for 48 h. The MIC was defined as the lowest extract concentration that completely inhibited visible bacterial growth. To determine the MBC, 10 µL aliquots were aspirated from all wells showing absence of visible turbidity and subcultured onto fresh, unsupplemented MHA or BHI agar plates. Plates were incubated under appropriate atmospheric conditions (37°C for 24 h for *Staphylococci*; 37°C for 48 h anaerobically for *C. acnes*). The MBC was identified as the lowest extract concentration demonstrating $\geq 99.9\%$ bactericidal reduction relative to the initial inoculum. Assays were conducted in triplicate across three independent experiments.

In-vitro Anti-Inflammatory Assay: Inhibition of PGE₂ Production: To determine the capacity of the extracts to modulate key mediators of inflammatory hyperalgesia, suppression of PGE₂ production was quantified in lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophage cells according to established protocols¹⁵. Macrophages were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere of 5% CO₂. Cells were seeded into 24-well plates (2×10^5 cells/well) and pre-incubated for 24 h. Cell viability under extract exposure was verified prior to testing via the MTT assay, confirming that extract concentrations up to 1.50 mg/mL maintained $> 90\%$ cellular viability. Cells were co-treated with LPS (1.0 µg/mL) and graded concentrations of *L. japonicus* aerial or root extracts (0.25, 0.50, 1.00, and 1.50 mg/mL) for 24 h. Untreated cells served as normal controls, while cells exposed to LPS without plant extract served as the stimulated vehicle control. Supernatants were harvested, cleared by centrifugation at $2000 \times g$ for 10 min at 4°C, and preserved at -80°C. The extracellular concentration of PGE₂ was measured using a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, Ann Arbor, MI, USA) in strict adherence to manufacturer instructions. Absorbance was read at 405 nm using a microplate reader, and PGE₂ concentrations were interpolated from a standard curve (3.9–500 pg/mL).

Statistical Analysis: All experimental analyses were performed in triplicate ($n = 3$), and data are presented as the mean $\pm$ standard deviation (SD). Statistical evaluations were conducted using SPSS software version 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 8.0. Differences between the two botanical parts (aerial vs. roots) were assessed using an independent two-tailed Student's t-test. Comparisons across multiple treatment concentrations in the PGE₂ assay were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc honest significant difference (HSD) test. Statistical significance was designated at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

RESULTS:

Phytochemical Component and Antioxidant Activity: The extraction yields for hot-water decoctions were $14.22 \pm 0.85\%$ (w/w) for aerial parts and $8.45 \pm 0.52\%$ (w/w) for roots. The quantitative yields of secondary metabolites (TPC and TFC) and corresponding antioxidant capacities (DPPH and FRAP) are summarized in **Table 1**.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT CAPACITIES OF HOT-WATER EXTRACTS OF *LEONURUS JAPONICUS* AERIAL PARTS AND ROOTS

Plant Part	Total phenolic content(mg GAE/g extract)	Total flavonoid content (mg QE/g extract)	DPPH Radical Scavenging(mgTE/g extract)	FRAP Reducing Power(mg TE/g extract)
Aerial parts	26.35 ± 0.11a	17.89 ± 0.19a	97.14 ± 0.15a	90.20 ±0.35a
Roots	7.40 ± 0.37b	5.77 ± 0.14b	18.03 ± 0.27b	20.61 ± 0.12b
t-statistic	78.53	89.21	443.20	340.85
p-value	< 0.001	< 0.001	< 0.001	< 0.001

Data are expressed as mean ± standard deviation of three independent replicates (n = 3). Means within the same column sharing different superscript letters (a, b) indicate statistically significant differences between aerial and root extracts (p < 0.001, independent Student's t-test). Abbreviations: GAE, Gallic Acid Equivalents; QE, Quercetin Equivalents; TE, Trolox Equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, Ferric Reducing Antioxidant Power.

Antibacterial Potential against Acne-Associated Bacteria: The *in-vitro* antibacterial screening of *L. japonicus* extracts revealed distinct, differential inhibitory activities against the three tested bacterial species **Table 2**.

TABLE 2: MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF *LEONURUS JAPONICUS* EXTRACTS AGAINST ACNE-INDUCING BACTERIA

Plant Part	Minimum inhibitory concentration (MIC,µg/mL)			Minimum bactericidal concentration (MBC,µg/mL)		
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>C. acnes</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>C. acnes</i>
Aerial parts	1250.0	1250.0	625.0	2500.0	2500.0	1250.0
Roots	2500.0	1250.0	1250.0	5000.0	5000.0	1250.0
Reference standard*	≤ 1.0	≤ 0.5	≤ 0.25	≤ 2.0	≤ 1.0	≤ 0.5

* Reference standard values represent published benchmark susceptibility ranges for standard antibiotics (e.g., Clindamycin / Erythromycin) ^{13, 16}. Assays were performed in triplicate (n = 3). Strains tested: *Cutibacterium acnes* (ATCC 6919), *Staphylococcus aureus* (ATCC 25923), and *Staphylococcus epidermidis* (ATCC 12228).

In-vitro Anti-Inflammatory Activity: PGE₂ Suppression: Evaluation of inflammatory mediator secretion indicated that uninduced macrophages maintained basal PGE₂ levels at 124.5 ± 15.2 pg/mL, which surged markedly following LPS challenge ($1.0 \mu\text{g/mL}$) to 1985.6 ± 48.2 pg/mL (p < 0.001). Co-incubation with aqueous extracts of *L. japonicus* resulted in a concentration-dependent downregulation of PGE₂ release across the tested range ($0.25\text{--}1.50 \text{ mg/mL}$) **Table 3**.

TABLE 3: EFFECT OF *LEONURUS JAPONICUS* EXTRACTS ON PROSTAGLANDIN E₂ (PGE₂) RELEASE IN LPS-STIMULATED RAW 264.7 MACROPHAGES

Experimental Treatment Group	Extract Conc. (mg/mL)	PGE ₂ Concentration (pg/mL)	Inhibition Relative to LPS Control (%)
Normal Control (Unstimulated)	—	124.5 ± 15.2	—
LPS Control (1.0 µg/mL)	—	1985.6 ± 48.2	0.0%
Aerial Parts Extract + LPS	0.25	1459.0 ± 125.4 **	26.5%
	0.50	1342.0 ± 82.1 ***	32.4%
	1.00	725.0 ± 85.6 ***	63.5%
	1.50	481.0 ± 88.5 ***	75.8%
Root Extract + LPS	0.25	1970.0 ± 65.0 (ns)	0.8%
	0.50	1730.0 ± 54.2 *	12.9%
	1.00	1120.0 ± 26.5 ***	43.6%
	1.50	968.0 ± 34.2 ***	51.2%

Data represent mean ± SD (n = 3). Statistical significance compared to the LPS-induced control group: ns = non-significant (p > 0.05), * p < 0.05, ** p < 0.01, *** p < 0.001 (one-way ANOVA followed by Tukey's post-hoc test).

DISCUSSION: Traditional Chinese Medicine documentation and historical pharmacopoeias have consistently prioritized the aerial biomass of *L. japonicus* (*Herba Leonuri*) over subterranean roots for clinical preparations^{1, 2}. The present comparative experimental findings substantiate this ancient empirical preference on modern phytochemical and pharmacological grounds.

In the present investigation, hot-water extraction was deliberately selected over conventional organic solvents (such as methanol, ethanol, or ethyl acetate). While lipophilic and medium-polarity organic solvents routinely yield higher concentrations of specific non-polar diterpenoids¹⁷, aqueous decoctions reflect the exact modality of traditional human consumption (tang ji) and ensure an extract devoid of hazardous solvent residues¹⁸. Water at elevated temperatures (100°C) disrupts plant cellular matrices, increasing the solubility and mass transfer of polar polyphenols, flavonoid glycosides, and water-soluble alkaloids¹⁸.

Our quantitative results revealed that the aerial parts of *L. japonicus* cultivated in Northern Thailand possess substantial quantities of phenolics (26.35 ± 0.11 mg GAE/g) and flavonoids (17.89 ± 0.19 mg QE/g), surpassing root values by 3.5-fold and 3.1-fold, respectively. The pronounced disparity in secondary metabolite accumulation between anatomical parts is consistent with botanical biosynthetic partitioning: photosynthetic tissues and inflorescences are direct sites of secondary metabolite synthesis driven by solar ultraviolet radiation and defense requirements, whereas roots primarily serve structural anchorage and nutrient uptake functions^{11, 19}.

The higher phenolic and flavonoid content in aerial structures correlated directly with superior radical-scavenging and reducing capacity. In the DPPH assay, the aerial extract demonstrated 97.14 ± 0.15 mg TE/g, and in the FRAP assay, 90.20 ± 0.35 mg TE/g. Polyphenolic compounds serve as effective hydrogen donors and electron transfer agents, neutralizing chain-carrying peroxy radicals and reducing reactive transition metals^{12, 19}. When contextualized with published data on other Lamiaceae members, such as *Leonurus cardiaca*¹¹ and *Leonurus sibiricus*²⁰, the aqueous aerial extract of *L. japonicus* exhibits comparable antioxidant

magnitude, establishing its utility as an accessible source of water-soluble radical-scavenging constituents.

Acne vulgaris pathogenesis is intrinsically linked to follicular microbial proliferation, accompanied by localized oxidative stress and tissue inflammation^{6, 7}. *C. acnes* triggers follicular rupture and inflammatory signaling via toll-like receptors, while opportunistic *S. epidermidis* and *S. aureus* contribute to secondary biofilm formation and superficial pustule development^{7, 21}. The broth microdilution data demonstrated that *L. japonicus* aerial extracts exerted moderate antimicrobial activity, exhibiting the strongest inhibition against *C. acnes* (MIC 625.0 µg/mL, MBC 1250.0 µg/mL) and twofold higher values against *S. aureus* and *S. epidermidis* (MIC 1250.0 µg/mL). Root extracts were less effective (MIC 1250–2500 µg/mL).

It must be acknowledged that compared to commercial pharmaceutical antibiotics such as clindamycin or erythromycin, which typically exhibit MICs below 1.0 µg/mL against susceptible bacterial strains^{13, 16}, the inhibitory concentrations observed for these crude aqueous extracts fall into the moderate range typical of unpurified botanical decoctions²². Phenolic compounds and flavonoid aglycones present in Lamiaceae species are documented to permeabilize bacterial plasma membranes, dissipate proton motive forces, and disrupt cell wall architecture^{22, 23}. While crude aqueous extracts cannot substitute for potent antibiotic monotherapies, their combined antibacterial and antioxidant profile suggests potential utility as topical adjunctive agents, particularly in light of expanding antimicrobial resistance among cutaneous microflora.

In the cellular assay, both extracts produced a concentration-dependent reduction in PGE₂ production in LPS-stimulated RAW 264.7 macrophages without compromising cellular viability. Prostaglandin E₂ is a central eicosanoid mediator synthesized via the arachidonic acid cascade through the rate-limiting enzymatic activity of cyclooxygenase-2 (COX-2) and inducible microsomal prostaglandin E synthase-1 (mPGES-1)^{9, 15}. Pathologically elevated PGE₂ orchestrates peripheral pain sensitization by lowering the activation threshold of primary

nociceptive neurons and driving downstream vasodilation and edema^{8,9}.

Earlier studies on *L. japonicus* have predominantly focused on its major pure alkaloid, leonurine, which suppresses NF- κ B and MAPK signaling cascades, thereby downregulating inducible nitric oxide synthase (iNOS) and COX-2 gene transcription^{24, 25}. The significant, dose-dependent decrease in PGE₂ elicited by the crude aerial extract (reaching 75.8% inhibition at 1.50 mg/mL) indicates that hot-water decoctions effectively extract these bioactive polar alkaloids and flavonoids.

Although historical clinical applications of Yi-Mu-Cao frequently address primary dysmenorrhea and pelvic pain conditions pathologically characterized by endometrial sloughing, excessive uterine prostaglandin accumulation (PGE₂ and PGF_{2 α}), and myometrial hypercontractility^{8, 9, 26} direct extrapolation from the present in vitro macrophage model to clinical dysmenorrhea must be avoided. The present data demonstrate cellular PGE₂ suppression in a murine macrophage system; whether this translates to reduced uterine contractility or clinical analgesia requires rigorous *in-vivo* and clinical verification. Rather than declaring an “analgesic” cure, our findings provide biochemical evidence that hot-water extracts of *L. japonicus* exert multi-target *in-vitro* anti-inflammatory effects by attenuating prostaglandin hypersecretion.

Future studies should prioritize chromatographic characterization (e.g., HPLC-DAD-MS/MS) to quantify specific marker constituents such as leonurine, stachydrine, and rutin in these regional extracts, elucidate direct enzymatic inhibitory kinetics against COX-1 and COX-2, and validate these anti-inflammatory observations in primary uterine tissue models.

CONCLUSIONS: The present study provides a rigorous comparative evaluation of aqueous extracts derived from the aerial parts and roots of *Leonurus japonicus* cultivated in Northern Thailand. The aerial part decoction contained significantly greater total phenolic and flavonoid content, conferring superior DPPH and FRAP antioxidant capacities compared to the root system.

Furthermore, the aerial extract demonstrated moderate antibacterial action against acne-associated pathogens (*Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) and elicited a concentration-dependent reduction in pro-inflammatory prostaglandin E₂ (PGE₂) production *in-vitro*. These empirical findings provide scientific rationale for the preferential use of aerial biomass over roots in traditional herbal preparations and highlight the potential of aqueous *L. japonicus* extracts as botanical candidates for further anti-inflammatory and dermatological formulation research.

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CONFLICT OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this manuscript

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