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RESEARCH ARTICLE

Comparative bioactivities of leaf and stem extracts of *Houttuynia cordata* Thunb.: Antioxidant, anti-inflammatory and antibacterial properties

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Abstract

Houttuynia cordata Thunb. is a medicinal plant widely used in traditional medicine and known for its diverse bioactive secondary metabolites. However, differences in metabolite composition between plant tissues and their functional implications remain unclear. This study assessed whether tissue-specific secondary metabolite profiles in *H. cordata* leaves and stems are associated with differences in antioxidant, anti-inflammatory and antibacterial activities. The botanical identification of the plant material was confirmed by *matK* gene sequencing. Gas chromatography-mass spectrometry (GC-MS) analysis revealed tissue-specific phytochemical distribution, with leaves enriched in biologically active compounds, whereas stems contained a greater number of detected compounds, indicating higher chemical diversity based on compound distribution within *H. cordata*. Both extracts exhibited dose-dependent anti-inflammatory activity through inhibition of nitric oxide (NO) production in RAW 264.7 macrophages; the stem extract showed stronger inhibitory potency, while the leaf extract demonstrated higher cell viability. The leaf extract also consistently exhibited higher antioxidant capacity in 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assays. Furthermore, the leaf extract exerted stronger antibacterial activity and a broader inhibitory spectrum against *Cutibacterium acnes*, particularly among isolates from moderate to severe acne patients. These findings suggest that *H. cordata* may be associated with multiple biological effects related to bacterial growth, oxidative stress and inflammation, highlighting its potential relevance for developing herbal dermatological applications.

Keywords: acne vulgaris; anti-inflammatory; antioxidant; *Cutibacterium acnes*; Gas chromatography-mass spectrometry; *Houttuynia cordata*

Introduction

Plants synthesise diverse secondary metabolites that are often unevenly distributed among tissues, reflecting ecological specialisation, including protection against microbes, herbivores and oxidative stress (1, 2). Understanding tissue-specific metabolite distribution is important for identifying plant-derived bioactive compounds with functional applications. This concept is particularly relevant in acne, a multifactorial skin disorder involving excessive sebum production, follicular hyperkeratinisation and rogen-mediated hormonal imbalance, microbial dysbiosis and chronic inflammation. Skin-associated bacteria including *Cutibacterium acnes* represent a relevant model to evaluate selective antimicrobial effects (3, 4).

In addition to bacterial colonisation, acne inflammation is closely associated with oxidative stress characterised by elevated reactive oxygen species (ROS) at lesion sites. Reactive oxygen species not only damage keratinocytes and follicular structures but also stimulate the release of pro-inflammatory cytokines, thereby aggravating disease severity (5, 6). Current treatments mainly rely on topical and systemic antibiotics including clindamycin, erythromycin, doxycycline and tetracycline. However, the increasing prevalence of antibiotic-resistant *C. acnes* strains has reduced therapeutic efficacy and created a global dermatological challenge (7, 8). Moreover, conventional antibiotics often fail to adequately address oxidative stress and persistent inflammation, which are major contributors to tissue damage and scarring. These limitations highlight the need for alternative multi-target therapeutic strategies.

Houttuynia cordata Thunb. is a medicinal plant rich in flavonoids, volatile aldehydes and polyphenols. Previous studies have reported antimicrobial and anti-inflammatory activities of *H. cordata*, but the potential functional differences among plant tissues remain poorly understood. Additionally, its phytochemicals exhibit anti-inflammatory effects by suppressing pro-inflammatory cytokines and strong antioxidant activity by scavenging free radicals and protecting skin cells from damage (9, 10). Despite these promising properties, comparative evidence regarding different

plant tissues and their activity against clinical *C. acnes* isolates from varying acne severities remains limited.

Therefore, this study aimed to compare leaves and stems of *H. cordata* to determine whether tissue-specific phytochemical composition translates into differential antioxidant capacity, anti-inflammatory activity and selective inhibition of skin-associated bacterial isolates.

Materials and Methods

Collection and authentication of the plant material

H. cordata plant was collected from Vinh Long, Vietnam.

Preparation of plant extracts

Fresh *H. cordata* collected from Vinh Long, Vietnam was washed, dried at 50 °C, powdered and macerated in ethanol (1:10 w/v) for 72 hr. Filtrate was evaporated under reduced pressure and freeze-dried. Extraction yield (%) was calculated.

$$\text{Extraction yield (\%)} = \frac{\text{Extract weight}}{\text{Dry powder weight}} \times 100$$

Study population

Skin swabs and comedones

Samples were collected from 30 acne patients and 15 healthy volunteers. Participants were divided into 3 groups: healthy controls (n = 15), mild acne (n = 15) and moderate-severe acne (n = 15). Acne severity was classified according to the American Family Physician and DermNet New Zealand criteria (11). Subjects had not used topical or systemic antibiotics (12), probiotics, or prebiotics at the time of sampling. Individuals with immunosuppressive therapy, systemic diseases or refusal to participate were excluded. All participants voluntarily agreed to participate in the study and provided informed consent prior to sample collection. Samples were collected using non-invasive procedures and anonymised before analysis. This study was approved by the Institutional Ethics Committee of Can Tho University (Approval No: Ref.No.24.004/PCT-HĐĐĐ). Written informed consent was obtained from all participants prior to sample collection.

Isolation and cultivation of *Cutibacterium acnes*

Samples were collected from 30 acne patients (comedone contents) and 15 healthy individuals (facial sebum). Specimens were enriched in 5 mL fluid thioglycollate medium (FTM) at 37 °C for 14 days, serially diluted (10^{-1}–10^{-3}) and spread onto Schaedler agar plates followed by anaerobic incubation at 37 °C for 14 days. Representative colonies were repeatedly sub-cultured until pure isolates were obtained (13). A total of 74 bacterial isolates were identified to the species level by amplification and sequencing of the 16S rRNA gene. *Cutibacterium acnes* isolates were used for antibacterial assays in this study.

Molecular identification

Total DNA was extracted using cetyltrimethylammonium bromide (CTAB) method (14). The *matK* region was amplified using primers *matK*-4600 and trnK-2R under polymerase chain reaction (PCR) conditions and sequenced for species confirmation (15). Molecular identification using the *matK* gene was performed to ensure accurate species authentication and reproducibility, particularly in phytochemical and bioactivity studies where misidentification of plant material may significantly affect the interpretation of results.

Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using an Agilent 7890B gas chromatograph coupled with an Agilent 5977A mass selective detector (MSD). The system was equipped with a DB-5MS capillary column (30 m × 0.25 mm i.e., 0.50 µm film thickness) coated with 5 % phenyl-methyl polysiloxane. Sample preparation was conducted as follows: 5 g of dried ethanol extract from leaves or stems of *H. cordata* was dispersed in 50 mL distilled water and successively partitioned six times with 50 mL chloroform ($CHCl_3$). The $CHCl_3$ fraction was dried over anhydrous sodium sulfate (Na_2SO_4) and concentrated under reduced pressure. The residue was re-dissolved in $CHCl_3$ to obtain a final concentration of 5 mg/mL and filtered through a 0.22 µm membrane prior to analysis. Helium (He) was used as the carrier gas. A 1 µL sample was injected in split mode with a split ratio of 1:10. The injector temperature was set at 250 °C. The oven temperature program was initiated at 60 °C, increased at a rate of 5 °C/min to 280 °C and held at this temperature for 15 min. Mass spectrometric detection was carried out at 70 eV under electron ionisation mode, with a scan range of 50–1000 m/z. The detector temperature was set at 250 °C. Compounds were tentatively identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) mass spectral library and relative abundances were calculated based on peak area percentages.

Antioxidant assays

Antioxidant activity of the extract was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assays as previously described with slight modifications (16–18). Absorbance was measured using a UV–Vis spectrophotometer at 517 nm (DPPH), 734 nm (ABTS) and 593 nm (FRAP). Ascorbic acid and trolox were used as positive controls. Extracts were tested at concentrations of 80–10000 µg/mL.

Anti-inflammatory activity

RAW 264.7 macrophages were cultured in DMEM at 37 °C with 5 % CO_2 and seeded at 2×10^6 cells/mL in 96-well plates. Cells were pre-treated with extracts at different concentrations for 2 hr and stimulated with LPS (1 µg/mL) for 24 hr. Dexamethasone (0.8–100 µM) was used as a positive control. Nitric oxide (NO) production was determined using the Griess method and absorbance was measured at 540 nm using a microplate reader. Cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (19–22).

Antibacterial activity

Clinical isolates of *C. acnes* (10^8 CFU/mL) were incubated anaerobically with extracts (0.156–10 mg/mL) for 72 hr. Viability was determined by a modified MTT assay (23, 24). DMSO (10%) was used as the solvent control, while culture medium without treatment served as the blank control. All experiments were performed in triplicate.

Statistical analysis

All experiments were performed in triplicate (technical replicates) and results are expressed as mean ± standard deviation (SD). IC_{50} values were calculated using non-linear regression analysis with an online IC_{50} calculator (AAT Bioquest). EC_{50} values were calculated using Table Curve 2D. Statistical analysis was performed using one-way analysis of variance (ANOVA) and differences were considered statistically significant at $p \leq 0.05$.

Results and Discussion

Species identification

The *matK* gene sequencing results confirmed that the plant sample corresponded to *H. cordata*. Basic local alignment search tool (BLAST) comparison against the National Center for Biotechnology Information (NCBI) database showed a sequence identity of 99.91 % with reference chloroplast and plastid genomes (MN541092.1; PP109135.1) (Table 1).

Extraction yield

The ethanol extraction yielded 130.43 g of stem extract and 68.16 g of leaf extract, corresponding to extraction efficiencies of 16.78 % and 5.90 %, respectively. The higher yield obtained from stems indicates a greater proportion of ethanol-soluble constituents compared to leaves.

Gas chromatography-mass spectrometry profiling of volatile constituents in *Houttuynia cordata*

The GC-MS chromatograms of leaf (LDC) and stem (TDC) extracts are presented in Fig. 1. A total of 9 compounds were identified in LDC, whereas 37 compounds were detected in TDC (Supplementary Table S1 and S2). Compounds were tentatively identified by comparison with the NIST mass spectral library and similarity percentages are presented in Supplementary Tables S1 and S2. The total ion chromatogram (TIC) profile of TDC showed a broader distribution and higher peak density, indicating a greater number of detected compounds and thus higher chemical diversity in stem tissue.

Both extracts contained fatty acid esters (ethyl hexadecanoate, ethyl linoleate) and long-chain terpenoids. However, distinct tissue-specific compounds were observed. Phytol (0.7 %) and loliolide (0.3 %) were detected exclusively in LDC, while sulfur-containing compounds and furan derivatives predominated in TDC.

Phytol is a diterpene alcohol known for anti-inflammatory and antimicrobial activities (25). Loliolide, a monoterpene lactone derived from carotenoid oxidation, has been reported to exhibit antioxidant and immune-modulatory properties (26). The exclusive presence of these compounds in leaves suggests that they may contribute to the stronger biological activities observed in the leaf extract.

Fatty acid esters detected in both tissues have been reported to enhance dermal permeability and provide mild antimicrobial and soothing effects (27). Notably, LDC contained linolenate derivatives, whereas TDC contained oleate derivatives, indicating differences in lipid composition between tissues. Although the stem extract exhibited a greater number of detected compounds, the leaf extract was enriched with specific bioactive constituents. This tissue-specific accumulation of secondary metabolites may be associated with the superior antioxidant and antibacterial (*C. acnes*) activities observed in LDC and may support the preferential use of leaves for dermatological applications of *H. cordata* (28).

Antioxidant activity of *Houttuynia cordata* extracts

The LDC exhibited markedly stronger antioxidant activity than the stem extract TDC in DPPH, ABTS and FRAP assays. In the DPPH assay, LDC showed consistently higher radical scavenging capacity across all concentrations, with an EC_{50} of 41.46 ± 4.04 µg/mL compared to 212.01 ± 18.38 µg/mL for TDC. Similar trends were observed in ABTS and FRAP assays, where LDC presented EC_{50} values of 55.69 ± 4.10 µg/mL and 145.22 ± 9.69 µg/mL, respectively, markedly lower than TDC (ABTS: > 500 µg/mL; FRAP: 489.81 ± 18.09 µg/mL). Overall, TDC exhibited weak to moderate and assay-dependent antioxidant activity (Table 2).

The superior activity of LDC may be attributed to its higher content of polyphenols and flavonoids. Elevated levels of quercitrin, hyperoside and total phenolics in *H. cordata* leaves compared with stems, are consistent with stronger antioxidant capacity (29). This agrees with the established correlation between phenolic content

Table 1. Sequence similarity of the studied leaf sample against the NCBI database

Sl.No.	Reference species	Identity (%)	Accession number
1	*Houttuynia cordata* chloroplast, complete genome	99.91 %	MN541092.1
2	*Houttuynia cordata* plastid, complete genome	99.91 %	PP109135.1

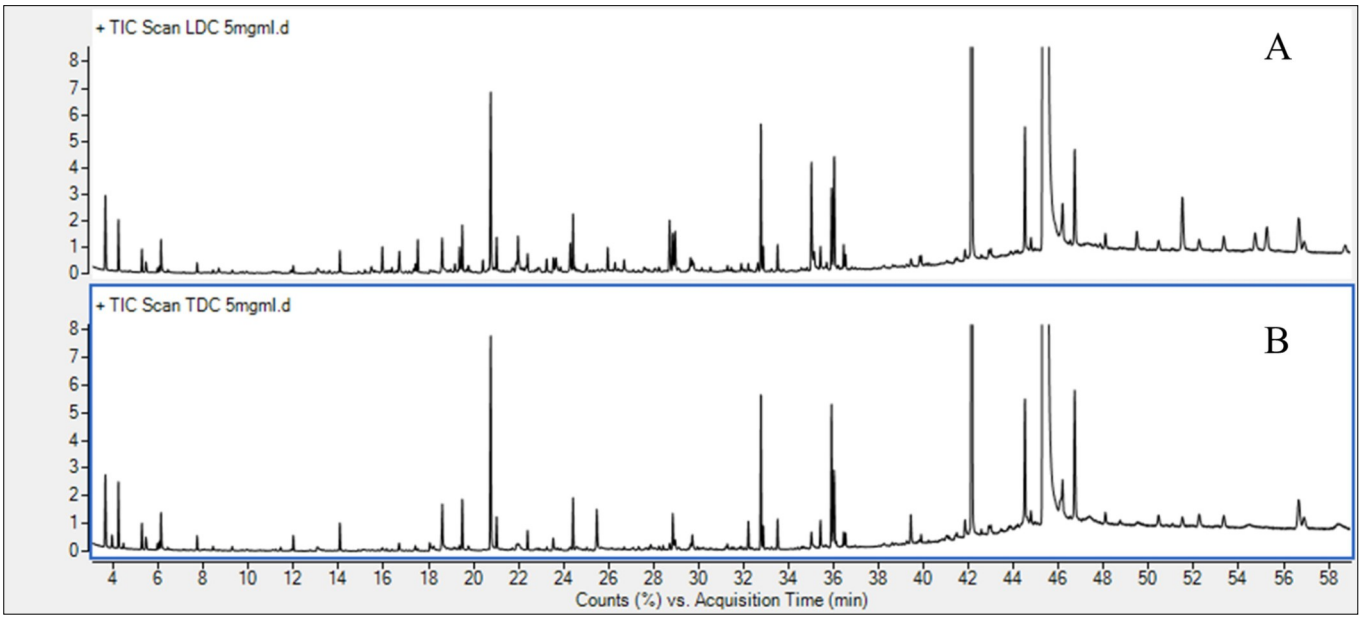


Fig. 1. Gas chromatography-mass spectrometry chromatograms of *Houttuynia cordata* leaves and stems.

Table 2. Antioxidant activity of *Houttuynia cordata* extracts

DPPH assay				
Concentration (µg/mL)	**LDC**	**TDC**	**Concentration (µg/mL)**	**L-Ascorbic acid (positive standard)**
500	79.63 ± 2.01	67.25 ± 2.35	100	92.32 ± 1.83
100	66.58 ± 1.44	42.39 ± 1.45	20	73.07 ± 1.28
20	35.98 ± 1.80	3.28 ± 0.28	4	32.58 ± 1.12
4	2.40 ± 0.21	-2.98 ± 0.23	0.8	12.71 ± 0.94
EC_{50}	41.46 ± 4.04	212.01 ± 18.38	EC_{50}	7.87 ± 0.57
ABTS assay				
Concentration (µg/mL)	**LDC**	**TDC**	**Concentration (µg/mL)**	**Trolox (positive standard)**
500	76.12 ± 1.24	39.32 ± 1.30	100	94.66 ± 2.06
100	61.95 ± 1.14	27.14 ± 1.16	20	70.48 ± 1.19
20	30.61 ± 1.06	6.55 ± 0.35	4	42.52 ± 1.03
4	7.59 ± 0.73	1.50 ± 0.14	0.8	13.80 ± 1.36
EC_{50}	55.69 ± 4.10	> 500	EC_{50}	6.63 ± 0.53
FRAP assay				
Concentration (µg/mL)	**LDC**	**TDC**	**Concentration (µg/mL)**	**L-Ascorbic acid (positive standard)**
500	75.46 ± 1.64	50.77 ± 1.39	100	91.00 ± 2.45
100	39.63 ± 1.27	14.89 ± 1.05	20	76.35 ± 1.41
20	11.42 ± 0.96	3.64 ± 0.12	4	34.61 ± 1.19
4	2.79 ± 0.24	1.03 ± 0.11	0.8	12.70 ± 0.91
EC_{50}	145.22 ± 9.69	489.81 ± 18.09	EC_{50}	7.35 ± 0.61

LDC: leaf extract; TDC: stem extract.

and radical scavenging activity (29, 30). Although weaker than L-ascorbic acid and trolox, the antioxidant activity of LDC remains significant for a natural extract and comparable to previously reported *H. cordata* extracts (31–33). Since oxidative stress contributes to acne initiation (34), these findings suggest that *H. cordata* leaf extract may help mitigate acne-associated inflammation through free-radical scavenging mechanisms.

Anti-inflammatory activity of *Houttuynia cordata* extracts

Both LDC and TDC extracts inhibited NO production in a concentration-dependent manner (Table 3). Increasing concentrations from 0.8–100 µg/mL resulted in progressively higher inhibition. At equivalent concentrations, TDC consistently showed stronger activity than LDC; at 100 µg/mL, inhibition reached 77.06 ± 2.00 % for TDC compared to 72.26 ± 2.58 % for LDC. The IC_{50} of TDC (37.09 ± 3.25 µg/mL) was lower than that of LDC (47.41 ± 3.89 µg/mL). Although both were less potent than dexamethasone (12.89 ± 1.39 µg/mL), they remained within a biologically relevant range.

Cell viability assays indicated higher biocompatibility for LDC than TDC at 100 µg/mL (96.12 ± 1.90 % and 86.20 ± 2.88 %), comparable to dexamethasone (97.25 ± 1.60 %). Both extracts maintained ≥ 86 % viability at the highest tested concentration, suggesting acceptable cytocompatibility.

These findings agree with the previous studies, reporting anti-inflammatory mechanisms of *H. cordata*, including modulation of PLA2, RAGE/TGF-β1, AKT/NF-κB, TLR4/NF-κB and TGF-β/Smad signaling pathways, as well as reduced TNF-α and IL-6 expression and inhibition of NF-κB activation (35–38). Compared with other herbal extracts, the NO-inhibitory activity of *H. cordata* can be considered moderate to strong and superior to many commonly used herbal teas (19, 21).

Antibacterial activity against *Cutibacterium acnes*

The ethanolic extracts of *H. cordata* exhibited concentration-dependent antibacterial activity against *C. acnes* isolates obtained from healthy individuals and acne patients. At low concentrations (≤ 0.3125 mg/mL), bacterial viability remained high and occasionally exceeded the untreated control, suggesting adaptive metabolic responses. Increasing concentrations (≥ 0.625 mg/mL) progressively reduced bacterial survival across most isolates.

The LDC generally showed stronger inhibitory activity than TDC, particularly at concentrations ≥ 2.5 mg/mL, where bacterial survival frequently decreased below 50 %. However, marked variability in susceptibility was observed among isolates, indicating strain-dependent sensitivity rather than uniform bactericidal activity.

Mean IC_{50} values were comparable among clinical groups but showed substantial dispersion (Table 4). Healthy-skin isolates demonstrated weak susceptibility, whereas acne-associated isolates displayed greater variability and occasional high sensitivity. Several moderate-severe acne isolates showed strong inhibition (IC_{50} < 1–4 mg/mL), while many healthy isolates exhibited minimal inhibition (IC_{50} > 10 mg/mL).

Table 3. Inhibitory effects of extracts on nitric oxide (NO) production

Concentration (µg/mL)	LDC % NO inhibition	LDC % cell viability	TDC % NO inhibition	TDC % cell viability	Dexamethasone % NO inhibition	Dexamethasone % cell viability
100	72.26 ± 2.58	96.12 ± 1.90	77.06 ± 2.00	86.20 ± 2.88	88.55 ± 1.32	97.25 ± 1.60
20	30.67 ± 1.26	98.49 ± 0.43	37.97 ± 1.55	94.06 ± 2.63	54.96 ± 1.24	99.22 ± 1.73
4	15.39 ± 1.05	—	18.89 ± 1.05	—	40.97 ± 1.04	—
0.8	2.10 ± 0.15	—	8.70 ± 0.66	—	33.18 ± 1.06	—
IC_{50} (µg/mL)	47.41 ± 3.89	—	37.09 ± 3.25	—	12.89 ± 1.39	—

LDC: leaf extract; NO: nitric oxide; TDC: stem extract.

Detailed strain-level responses are provided in Supplementary Tables S3–S5 and Supplementary Fig. S1–S6.

The antibacterial activity of *H. cordata* observed in this study appears selective rather than broadly bactericidal. This selective inhibitory pattern suggests a potential role in modulating the skin microbiome rather than complete eradication of *C. acnes*. Maintaining microbiome balance is increasingly recognised as an important strategy in acne management, as disruption of commensal populations may contribute to dysbiosis and disease progression. The large inter-strain variability suggests that susceptibility depends on physiological adaptation and metabolic characteristics rather than species identity alone. Genomic studies indicate that antimicrobial sensitivity in *C. acnes* is primarily influenced by metabolic pathways, stress-response systems and genome structural variation rather than classical virulence determinants (13, 39, 40).

The stronger activity of the leaf extract is consistent with its higher content of flavonoids and bioactive metabolites. Previous studies demonstrated that ethanol extracts of *H. cordata*, enriched in phenolic compounds, inhibit the growth of *C. acnes* more effectively than aqueous extracts (10). Specific flavonoids such as hyperin and quercitrin have also been reported to contribute to antibacterial activity (41, 42).

Importantly, acne-associated isolates showed higher susceptibility than commensal isolates. This pattern suggests a microbiome-modulating effect rather than indiscriminate antibacterial activity. Complete eradication of *C. acnes* may destabilise the skin microbiota and contribute to dysbiosis; therefore selective inhibition is considered advantageous in acne management (34).

Table 4. Comparison of antibacterial susceptibility of *Cutibacterium acnes* isolates to *Houttuynia cordata* extracts

Clinical origin	TDC IC_{50} (mg/mL)	LDC IC_{50} (mg/mL)
Healthy skin	8.76 ± 3.53	8.16 ± 3.98
Mild acne	9.29 ± 2.30	12.42 ± 19.91
Moderate–severe acne	9.33 ± 10.29	7.97 ± 10.80

LDC: leaf extract; TDC: stem extract.

Compared with other botanical preparations, the inhibitory activity observed here falls within the moderate to strong biological range and exceeds that reported for several herbal extracts with weak activity (19, 21). Previously reported antioxidant properties of *H. cordata*, showed a multimodal mechanism relevant to acne pathogenesis (29, 30).

Overall, *H. cordata* may not act as a conventional antibiotic but may exhibit selective inhibitory effects, suggesting a potential role in modulating the skin microbiome. This property may be relevant for the development of topical anti-acne applications, particularly in the context of increasing antibiotic resistance.

Conclusion

The present study suggests tissue-dependent functional differences in *Houttuynia cordata*. Leaf extracts exhibited stronger antioxidant and antibacterial effects, whereas stem extracts showed comparatively higher anti-inflammatory activity, indicating differences in the distribution of bioactive metabolites between plant tissues. These differences correspond with distinct phytochemical profiles revealed by GC-MS analysis, indicating variation in compound composition. Overall, the observed biological activities may reflect intrinsic physiological strategies of the plant. This study highlights the association between plant efficacy and tissue-specific phytochemical composition.

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Authors' contributions

XMT contributed to the conceptualisation of the study, development of software, data curation, formal analysis, interpretation of data and preparation of the original draft. PXH was involved in data curation, visualisation and assisted in the interpretation of data. MXN contributed to the investigation, sample collection, laboratory experiment and methodology development. ATHN performed formal analysis, validation, visualisation and contributed to data interpretation. TAHV and LKHV was responsible for critical revision of the manuscript, language editing and literature review. TNN contributed to data curation, visualisation and interpretation of results. DNT supervised the study and contributed to conceptualisation, methodology, investigation, validation, manuscript reviewing and editing. All authors contributed to the study conception and design, participated in data analysis or interpretation and review. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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