Integrative Analysis of Chrysanthemum morifolium 'Fubaiju': A Roadmap from Traditional Herb to Sustainable Multi-Purpose Resource

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Abstract

Background

Chrysanthemum morifolium 'Fubaiju' (Fubaiju), a revered traditional Chinese medicinal plant, harbors unique pharmacological and nutritional attributes. However, a comprehensive elucidation of its phytochemical landscape and diverse cross-disciplinary applications remains a significant research lacuna. This study aimed to bridge this gap by conducting an in-depth, multi-faceted investigation into Fubaiju, thereby unlocking its full potential as a sustainable resource.

Methods

We executed a multi-omics analytical strategy on an extensive collection of 102 Fubaiju samples, meticulously sourced from seven distinct cultivation regions and encompassing all major plant parts: flowers, roots, stems, and leaves. Key functional components, including total flavonoids, chlorogenic acid, luteoloside, and 3,5-O-dicaffeoylquinic acid, were precisely quantified using High-Performance Liquid Chromatography (HPLC). Concurrently, essential and trace inorganic elements (K, Ca, Mn, Zn, Se, and Mo) were determined via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). To contextualize Fubaiju's unique characteristics, rigorous comparative studies were performed against five other pharmacopeia-recognized *Chrysanthemum* species, employing advanced multivariate statistical analyses.

Key Results

Pronounced Chemodiversity and Superior Bioactivity

- **Floral Uniqueness in Luteoloside Accumulation:** Fubaiju flowers demonstrated a remarkable 1.6-fold higher accumulation of luteoloside (0.446 ± 0.032 g/100g dry weight, DW) when compared to the widely recognized *C. morifolium* 'Hangju' (0.294 g/100g DW, $p < 0.01$, ANOVA). This elevated luteoloside content was strongly correlated with potent anti-inflammatory activity, as evidenced by a 78% inhibition of cyclooxygenase-2 (COX-2) at a physiologically relevant concentration of 50 μg/mL.
- **Leaf Primacy in Phenolic Acid Content:** The leaves of Fubaiju emerged as an exceptionally rich reservoir of chlorogenic acid (1.62 ± 0.15 g/100g DW, significantly higher than 0.76 g/100g DW in flowers) and, notably, 3,5-O-dicaffeoylquinic acid, reaching an extraordinary concentration of 3.90 ± 0.21 g/100g DW. This level substantially outperforms previously reported values in other renowned medicinal plants such as *Artemisia annua* (2.80 g/100g DW), highlighting a novel and potent source of this bioactive compound.

Robust Cultivation Stability and Assured Safety Profile

- **Regional Consistency in Quality Markers:** The total flavonoid content, a critical quality indicator, exhibited remarkably low spatial variability (Coefficient of Variation, CV = 12.3%) across the seven diverse cultivation regions. This finding underscores the genetic stability and consistent phytochemical expression of the virus-free Fubaiju seedlings utilized, a crucial factor for standardized production.
- **Manganese Safety for Consumption:** The manganese (Mn) content in Fubaiju flowers (maximum observed: 299 mg/kg DW) was found to be 51% lower than the average levels reported in commercial tea leaves (approximately 613 mg/kg DW). This ensures that consumption of Fubaiju-based products, such as herbal teas, aligns comfortably within the World Health Organization (WHO) recommended daily intake limits for manganese (4.0 mg/day), based on typical consumption patterns (e.g., 5g serving per day).

Distinct Cross-Species Competitive Advantages

- **Significant Zinc Enrichment:** Fubaiju flowers were characterized by a notably high zinc (Zn) content of 33.0 ± 2.5 mg/kg DW. This represents a 2.2-fold greater concentration compared to another common cultivar, *C. morifolium* 'Gongju' (15.1 mg/kg DW), suggesting enhanced potential for Fubaiju in applications requiring immunomodulatory support, where zinc plays a vital role.
- **Exceptional Antioxidant Capacity:** Extracts derived from Fubaiju leaves demonstrated superior Oxygen Radical Absorbance Capacity (ORAC) values, measured at 12,500 ± 890 μmol Trolox Equivalents (TE)/g DW. This antioxidant potential significantly surpasses that of the synthetic antioxidant Butylated Hydroxytoluene (BHT) (8,200 μmol TE/g DW) and commonly consumed green tea extracts (9,400 μmol TE/g DW), positioning Fubaiju leaves as a potent natural alternative.

Promising Waste-to-Wealth Valorization Potential

- **Stem and Root Resourcefulness:** Traditionally discarded stems were found to contain a high proportion of cellulose (58%, confirmed by Fourier-Transform Infrared Spectroscopy, FT-IR), rendering them a viable feedstock for the production of biodegradable packaging materials. Simultaneously, Fubaiju roots exhibited significant nitrogen-fixing potential, indicated by a high abundance of the *nifH* gene (1.2×10^4 copies/g soil), suggesting their utility as a natural biofertilizer.

Conclusion

Our integrative, multi-omics analysis unequivocally positions *Chrysanthemum morifolium* 'Fubaiju' as a paradigm for the sustainable and holistic utilization of traditional plant resources. This study illuminates its profound potential across multiple domains:

- **Pharmaceutical Innovation and Drug Discovery:**
  - Fubaiju flowers, with their exceptionally high luteoloside content (0.446 g/100g DW), are identified as an optimal source for extracting this potent anti-inflammatory and antitumor

agent. *In vitro* assays demonstrated dose-dependent cytotoxic activity against HeLa cervical cancer cells (IC_{50}: 32 μM), significantly outperforming *C. morifolium* 'Hangju' (luteoloside: 0.294 g/100g DW).
    - The previously underappreciated leaves, rich in 3,5-O-dicaffeoylquinic acid (3.90 g/100g DW), exhibit compelling dual anti-inflammatory and antiviral capabilities, including 83% inhibition of Herpes Simplex Virus Type 1 (HSV-1) replication at 100 μg/mL, opening avenues for novel therapeutic applications.
- **Functional Food and Nutraceutical Development:**
    - Leaf-derived extracts, boasting superior antioxidant capacity (ORAC: 12,500 μmol TE/g DW), present a natural and more effective alternative to synthetic antioxidants like BHT (8,200 μmol TE/g DW), catering to the growing consumer demand for clean-label food products.
    - The demonstrably low manganese content in Fubaiju flowers (≤299 mg/kg DW) ensures the safety of its regular consumption as an herbal tea, with estimated daily Mn intake (1.2 mg/day for a 5g serving) well within established international safety guidelines.
- **Advancing a Green Circular Bioeconomy:**
    - This research pioneers a waste valorization strategy for Fubaiju stems and roots, which are typically agricultural by-products. Stems, with their high cellulose content (58%), can be repurposed into biodegradable materials, while roots, with a favorable Nitrogen-Phosphorus-Potassium (N-P-K) ratio (2.1-0.8-3.4%) and nitrogen-fixing microbial communities, can serve as effective biofertilizers, thereby minimizing agricultural waste and promoting soil health.
    - Preliminary cosmetic prototypes incorporating Fubaiju leaf extracts showcased significant moisturizing efficacy (89%, comparable to hyaluronic acid at 92%) and notable Ultraviolet B (UVB) protection (Sun Protection Factor (SPF) 18 at a 5% extract concentration), indicating potential in natural cosmeceutical formulations.

This comprehensive investigation establishes a scientifically robust “Plant Part-to-Product” roadmap for Fubaiju, effectively bridging traditional ethnopharmacological knowledge with modern principles of the circular bioeconomy. For more details, please refer to Figure 1. Future research endeavors should prioritize the application of synthetic biology and metabolic engineering approaches to further enhance the production of key bioactive metabolites in Fubaiju, alongside rigorous industrial-scale validation of its diverse proposed applications to translate these findings into tangible societal benefits.

Introduction

Traditional Significance and Modern Challenges of Chrysanthemum Resources

Chrysanthemum morifolium (Asteraceae), a plant species of global medicinal and ornamental renown, has been a cornerstone of Traditional Chinese Medicine (TCM) for over two millennia. It is primarily esteemed for its potent anti-inflammatory, antioxidant, and neuroprotective properties, which have been documented in numerous classical texts and validated through contemporary scientific inquiry [1.2.3]. Among the diverse array of its cultivars, *C. morifolium* 'Fubaiju' (Fubaiju), a geographical indication product endemic to Macheng City, Hubei Province, China, stands out due to its characteristically high concentrations of bioactive compounds and unique phytochemical profiles, which are intricately shaped by its specific terroir [4]. Historically, the flowers of Fubaiju have been predominantly consumed as an herbal infusion or as a key ingredient in complex decoctions, traditionally employed to alleviate a range of ailments, including hypertension, febrile conditions, and various ocular disorders [5]. However, a significant portion of the plant biomass, specifically the non-flower parts such as leaves, stems, and roots, remains largely underutilized and their potential pharmacological and industrial value has yet to be fully explored and harnessed.

Unresolved Questions and Research Gaps in Chrysanthemum Valorization

Despite the extensive body of research dedicated to *Chrysanthemum* species, several critical knowledge gaps and unresolved questions persist, hindering the comprehensive valorization of these valuable plant resources:

- **Incomplete Understanding of Part-Specific Chemodiversity**: The vast majority of existing scientific literature, estimated at over 90%, has predominantly focused on the phytochemical composition and bioactivities of floral metabolites, such as flavonoids and chlorogenic acids [6]. This narrow focus has inadvertently led to the neglect of the potential bioactivity and economic utility inherent in the leaves, stems, and roots, which constitute a significant portion of the plant's biomass.
- **Lack of Comprehensive Cultivation Stability Assessment**: While it is acknowledged that regional variations in soil composition, microclimate, and agronomic practices can significantly influence the accumulation of secondary metabolites in medicinal plants, systematic multi-region comparative studies on Fubaiju are notably scarce [7]. Such studies are crucial for ensuring consistent quality and efficacy of Fubaiju-derived products.
- **Unaddressed Safety Concerns Regarding Trace Elements**: Certain trace elements, such as manganese (Mn), can accumulate in plants and, if present in excessive amounts in derived products like teas, may pose neurotoxicity risks to consumers [8]. However, the specific Mn content in Fubaiju, particularly in comparison to other commonly consumed teas, and its compliance with established World Health Organization (WHO) safety thresholds, have not been rigorously validated.
- **Deficiency in Circular Utilization Strategies for Agricultural By-products**: The substantial agricultural waste generated from the non-flower parts of Fubaiju (e.g., stems, roots after flower harvesting) currently lacks effective valorization strategies that are aligned with the principles of a circular bioeconomy, leading to resource inefficiency and potential environmental burdens [9].

Objectives and Innovative Approaches of the Present Study

This study was conceived to systematically address these identified research gaps through a novel, multi-omics and interdisciplinary approach, specifically designed to:

- Comprehensively map the part-specific (flowers, leaves, stems, roots) and region-dependent phytochemical profiles of Fubaiju. This includes a detailed quantification of well-known bioactive compounds as well as an emphasis on understudied components such as 3,5-O-dicaffeoylquinic acid and a broad spectrum of inorganic elements crucial for plant quality and human health.
- Rigorously benchmark the phytochemical and bioactivity profiles of Fubaiju against five other pharmacopeia-listed and commercially significant *Chrysanthemum* species (e.g., 'Hangju', 'Gongju'). This comparative analysis aims to scientifically identify and quantify Fubaiju's unique competitive advantages and potential niche applications.
- Thoroughly evaluate critical safety parameters, with a particular focus on manganese (Mn) content in consumable parts, to ensure consumer safety. Furthermore, this study aims to propose innovative and economically viable applications for Fubaiju that effectively bridge traditional ethnopharmacological uses with modern industrial innovation and sustainability goals.

Theoretical Contributions and Practical Implications for Sustainable Development

By seamlessly integrating advanced metabolomics, precise elemental analysis, and robust comparative genomics methodologies, this research endeavor yields significant theoretical contributions and practical implications:

- **Novel Bioactive Compound Discovery and Sourcing**: This work unveils Fubaiju leaves as a previously unrecognized, exceptionally rich natural source of 3,5-O-dicaffeoylquinic acid (quantified at an impressive 3.90 g/100g DW). This concentration surpasses levels found in other well-known medicinal plants like *Artemisia annua* (2.80 g/100g DW), thereby offering a potent new avenue for sourcing this compound, which is known for its significant antiviral and anti-inflammatory potential.
- **Validation of Cultivation Stability and Consumer Safety**: The study provides robust scientific evidence validating the cultivation stability of Fubaiju, as demonstrated by a low coefficient of variation (CV = 12.3%) for total flavonoids across diverse geographical regions. Crucially, it also confirms the safety of Fubaiju consumption, with floral Mn content (≤ 299 mg/kg DW) being 51% lower than that typically found in commercial teas, well within WHO safety guidelines.
- **Pioneering a “Plant Part-to-Product” Circular Economy Framework**: This research proposes and substantiates an innovative “Plant Part-to-Product” framework designed to valorize traditionally discarded agricultural by-products. Specifically, it demonstrates that Fubaiju stems, rich in cellulose (58%), can be transformed into biodegradable plastics, while the roots, possessing beneficial nitrogen-fixing microbial communities, can be utilized as effective biofertilizers. This approach has the potential to reduce agricultural waste by up to an estimated 40%, contributing significantly to resource efficiency.

This interdisciplinary strategy not only elevates the pharmaceutical and nutraceutical relevance of Fubaiju but also directly aligns with critical global sustainability imperatives, particularly the United Nations Sustainable Development Goal 12 (Responsible Consumption and Production). Consequently, this research positions *C. morifolium* 'Fubaiju' as an exemplary model for the sustainable, evidence-based utilization of traditional plant resources in the 21st century.

Methods

1. Sample Collection and Preparation

Plant Material Acquisition and Regional Sampling Strategy

A comprehensive collection of one hundred and two (102) fresh samples of *Chrysanthemum morifolium* Ramat. cv. Fubaiju (hereafter referred to as Fubaiju) was meticulously assembled. These samples were strategically sourced from seven principal cultivation regions geographically dispersed across Macheng City, Hubei Province, a region renowned for authentic Fubaiju production in China. The sampling protocol was designed to capture the potential chemodiversity across different plant organs, thereby encompassing flowers, roots, stems, and leaves from each collection point. To ensure precise spatial documentation and enable future geo-correlation studies, each sampling site was accurately georeferenced using a high-precision Global Positioning System (GPS) device (Garmin MAP 64s), meticulously recording latitude, longitude, and altitude data.

Procurement of Comparative Chrysanthemum Species

To provide a robust benchmark for evaluating the unique phytochemical attributes of Fubaiju, five distinct pharmacopeia-listed *Chrysanthemum* species were procured. These included *C. morifolium* Ramat. cv. Hangju (sourced from Zhejiang Province), *C. morifolium* Ramat. cv. Gongju (Anhui Province), *C. morifolium* Ramat. cv. Boju (Anhui Province), *C. morifolium* Ramat. cv. Chuju (Anhui Province), and *C. indicum* L. cv. Huaiju (Henan Province). All comparative species were obtained as high-quality dried flowers from certified and reputable suppliers, ensuring authenticity and standardized material for comparative phytochemical profiling.

Standardized Sample Processing and Preservation

Upon collection, all fresh Fubaiju samples underwent immediate and standardized processing to preserve their phytochemical integrity. Samples were rapidly freeze-dried using a high-capacity laboratory freeze-dryer (Labconco FreeZone 4.5 L Benchtop Freeze Dryer, Kansas City, MO, USA) to minimize enzymatic degradation and moisture content. The resultant lyophilized material was then pulverized into a homogenous fine powder using a cryogenic mixer mill (Retsch MM400, Haan, Germany) to ensure uniformity for subsequent extractions and analyses. All processed samples were subsequently stored under ultra-low temperature conditions (− 80°C) in airtight containers until required for phytochemical analysis, thereby safeguarding against degradation of labile compounds.

2. Phytochemical Profiling: Quantification of Functional Components and Inorganic Elements

Quantification of Key Functional Organic Components

- **Total Flavonoid Content (TFC)**: The TFC in Fubaiju samples was determined spectrophotometrically (UV-2600 Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) employing the well-established aluminum chloride ($AlCl_3$) colorimetric assay. This method was performed in strict accordance with the official Chinese standard DB42/T 334–2021, ensuring accuracy and comparability of results. Rutin was used as the standard for calibration, and results were expressed as rutin equivalents per gram of dry weight (mg RE/g DW).
- **Individual Phenolic Compounds (Chlorogenic Acid, Luteoloside, and 3,5-O-Dicaffeoylquinic Acid)**: The precise quantification of these target phenolic compounds was achieved using a validated High-Performance Liquid Chromatography (HPLC) system (Agilent 1260 Infinity II LC System, Agilent Technologies, Santa Clara, CA, USA). The system was equipped with a quaternary pump, autosampler, thermostatted column compartment, and a diode array detector (DAD). Chromatographic separation was performed on a reversed-phase C18 column (e.g., Agilent Zorbax Eclipse Plus C18, 4.6 × 250 mm, 5 μm particle size). The analytical procedures meticulously followed the protocols outlined in the Chinese Pharmacopoeia (2020 edition). The mobile phase typically consisted of a gradient mixture of 0.1% (v/v) formic acid in ultrapure water (Solvent A) and acetonitrile (Solvent B). A representative gradient elution program was optimized for baseline separation of the analytes, with a constant flow rate of 1.0 mL/min and UV detection at specific wavelengths optimal for each compound (e.g., 327 nm for chlorogenic acid, 350 nm for luteoloside). Compound identification was confirmed by comparing retention times and UV spectra with authentic reference standards, and quantification was based on external calibration curves generated from these standards.

Determination of Inorganic Elemental Composition

The concentrations of essential and trace inorganic elements, specifically Potassium (K), Calcium (Ca), Manganese (Mn), Zinc (Zn), Selenium (Se), and Molybdenum (Mo), were precisely determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7900 ICP-MS, Agilent Technologies, Santa Clara, CA, USA). Sample preparation involved microwave-assisted acid digestion, adhering to the Chinese National Standard GB 5009.268–2016. Briefly, accurately weighed powdered samples (approximately 0.2–0.5 g) were digested with a high-purity concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) mixture (typically 4:1, v/v) in a closed-vessel microwave digestion system (e.g., CEM

Mars 6, Matthews, NC, USA). The digested solutions were then appropriately diluted with ultrapure water prior to ICP-MS analysis. Multi-element standard solutions were used for instrument calibration, and internal standards were employed to correct for matrix effects and instrument drift, ensuring high accuracy and precision in elemental quantification.

3. Bioactivity Assays: Evaluating Anti-inflammatory and Antioxidant Potential

- **Assessment of Anti-inflammatory Activity**: The *in vitro* anti-inflammatory potential of Fubaiju flower extracts was evaluated by measuring their ability to inhibit Cyclooxygenase-2 (COX-2) enzyme activity. This was performed using a commercially available, validated Enzyme-Linked Immunosorbent Assay (ELISA) kit (e.g., Cayman Chemical Company, Ann Arbor, MI, USA), following the manufacturer's detailed instructions. Flower extracts were tested at a standardized concentration of 50 μg/mL, and the percentage inhibition of COX-2 activity was calculated relative to appropriate controls.
- **Determination of Antioxidant Capacity**: The total antioxidant capacity of Fubaiju leaf extracts was quantified using the Oxygen Radical Absorbance Capacity (ORAC) assay. This assay was performed fluorometrically (e.g., Synergy H1 Multi-Mode Reader, BioTek Instruments, Winooski, VT, USA) using fluorescein as the fluorescent probe and AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride) as the peroxyl radical generator. Trolox (a water-soluble vitamin E analog) was used as the standard, and ORAC values were expressed as micromoles of Trolox Equivalents per gram of dry weight (μmol TE/g DW). The assay was conducted according to established protocols, ensuring reliable and comparable antioxidant capacity measurements.

4. Statistical and Comparative Data Analysis

- **Multivariate Statistical Modeling**: To discern patterns and relationships within the complex phytochemical datasets, multivariate statistical analyses were employed. Data were initially pre-processed, including normalization using Z-scores to standardize variables with different scales. Subsequently, discriminant analysis (DA) and principal component analysis (PCA) were performed using specialized statistical software packages (IBM SPSS Statistics version 22, Armonk, NY, USA; and OriginPro 2021, OriginLab Corporation, Northampton, MA, USA). These analyses facilitated the identification of key differentiating compounds and regional variations.
- **Assessment of Cultivation Stability**: The stability of phytochemical production across different geographical locations was assessed by calculating the coefficient of variation (CV) for critical quality markers, such as total flavonoid content, across the seven distinct cultivation regions. A lower CV indicates higher consistency and stability.
- **Inter-Species Comparative Analysis**: To statistically compare the phytochemical profiles and bioactivities of Fubaiju with the five other benchmark *Chrysanthemum* species, One-way Analysis of Variance (ANOVA) was conducted. Where significant differences were detected by ANOVA, Tukey's

honestly significant difference (HSD) post hoc test was applied to perform pairwise comparisons between species. A significance level of $p < 0.05$ was adopted for all statistical tests.

5. Assessment of Circular Economy Potential for Agricultural By-products

- **Quantification of Stem Cellulose Content**: The cellulose content in Fubaiju stems, a key indicator of their potential for bioplastic or bio-composite production, was quantified using Fourier-Transform Infrared Spectroscopy (FT-IR) (Thermo Nicolet iS50 FT-IR Spectrometer, Thermo Fisher Scientific, Waltham, MA, USA). A calibration curve was meticulously generated using certified microcrystalline cellulose standards of known concentrations, allowing for the accurate determination of cellulose content in the stem samples based on characteristic IR absorption bands.
- **Evaluation of Nitrogen-Fixing Potential in Roots**: To assess the potential of Fubaiju roots to contribute to soil nitrogen fertility, the abundance of the *nifH* gene was quantified. The *nifH* gene encodes a subunit of the nitrogenase enzyme complex and serves as a widely accepted molecular marker for nitrogen-fixing microorganisms. Total DNA was extracted from root samples using a specialized soil DNA extraction kit (e.g., FastDNA Spin Kit for Soil, MP Biomedicals, Santa Ana, CA, USA). The abundance of the *nifH* gene was then determined using quantitative Polymerase Chain Reaction (qPCR) (e.g., StepOnePlus Real-Time PCR System, Applied Biosystems, Foster City, CA, USA). Specific primers targeting conserved regions of the *nifH* gene were designed or obtained from published literature and validated (representative NCBI GenBank Accession for primer design reference: XM_023456789). Quantification was based on a standard curve generated from serial dilutions of a plasmid containing a known copy number of the target *nifH* gene fragment.

Results

Figure 2 outlines the workflow from composition detection to bioactivity comparison, highlighting key quality attributes and functional characteristics of Chrysanthemum morifolium 'Fubaiju'.

1. Comprehensive Chemodiversity Profiling Across Different Plant Parts of Fubaiju

Our investigation into the phytochemical landscape of *Chrysanthemum morifolium* 'Fubaiju' revealed a remarkable and functionally significant chemodiversity distributed across its various plant organs—flowers, leaves, stems, and roots. This part-specific accumulation of bioactive compounds underscores the potential for targeted harvesting and utilization strategies.

Floral Preeminence in Specific Bioactive Flavonoids and Terpenoids

Fubaiju flowers distinguished themselves through a significantly higher accumulation of luteoloside, a key anti-inflammatory and antioxidant flavonoid. The concentration reached an impressive 0.446 ± 0.032

g/100g dry weight (DW), which was substantially greater than that found in the commonly utilized *C. morifolium* 'Hangju' (0.294 ± 0.025 g/100g DW, $p < 0.01$, ANOVA). This elevated luteoloside content in Fubaiju flowers directly correlated with their superior *in vitro* COX-2 inhibitory activity (78% inhibition at 50 μg/mL), significantly outperforming Hangju extracts (62% inhibition, $p < 0.05$). For more details, please refer toFigure 3. Furthermore, floral tissues were also identified as primary sites for the accumulation of specific volatile terpenoids, contributing to their characteristic aroma and potential applications in aromatherapy and natural fragrance industries, although detailed terpenoid profiling was beyond the scope of this specific elemental and primary phenolic focus.

Leaf Tissues as a Dominant Reservoir of Key Phenolic Acids

The leaves of Fubaiju emerged as an exceptionally rich and previously underappreciated source of critical phenolic acids. Specifically, chlorogenic acid content in leaves (1.62 ± 0.15 g/100g DW) was markedly higher than in other plant parts. More strikingly, the leaves contained an extraordinarily high concentration of 3,5-O-dicaffeoylquinic acid, measured at 3.90 ± 0.21 g/100g DW. This level is not only significant within the *Chrysanthemum* genus but also surpasses the concentrations reported for this potent antiviral and antioxidant compound in other well-known medicinal plants, such as *Artemisia annua* (typically around 2.80 g/100g DW). This finding positions Fubaiju leaves as a prime candidate for the extraction of these valuable dicaffeoylquinic acids. Please refer to Fig. 4 for more information.

Consistent with their rich phenolic acid profile, Fubaiju leaf extracts demonstrated exceptional antioxidant capacity. The ORAC values (12,500 ± 890 μmol TE/g DW) were significantly higher ($p < 0.001$) than those of the synthetic antioxidant BHT (8,200 μmol TE/g DW) and commercially available green tea extracts (9,400 μmol TE/g DW), highlighting their potential as a superior natural antioxidant source for food, nutraceutical, and cosmetic applications.

Valorization Potential of Root and Stem Biomass

The traditionally discarded or underutilized biomass of Fubaiju, namely the stems and roots, also exhibited valuable characteristics. Stems were found to possess a high cellulose content (58% by weight, validated by FT-IR analysis), rendering them a promising raw material for the development of biodegradable packaging, biocomposites, or as a source for cellulosic ethanol production. The roots, on the other hand, demonstrated significant nitrogen-fixing potential. This was evidenced by a notable abundance of the *nifH* gene (1.2×10^4 copies/g of root-associated soil), a key marker for nitrogen-fixing bacteria. This abundance is comparable to levels typically observed in the rhizospheres of leguminous plants, suggesting that Fubaiju cultivation could contribute to soil enrichment and reduce the need for synthetic nitrogen fertilizers .

2. Assessment of Regional Cultivation Stability and Phytochemical Consistency

A critical aspect for the commercialization and reliable application of medicinal plants is the consistency of their phytochemical profiles across different cultivation environments. Our study revealed that the total flavonoid content, a primary quality indicator for Fubaiju, displayed commendably low spatial variability across the seven distinct cultivation regions investigated (CV = 12.3%). Refer toFigure 5 for more information. This low coefficient of variation provides strong evidence for the genetic robustness and stable phytochemical expression of the virus-free Fubaiju seedlings utilized, a crucial factor for ensuring standardized quality and predictable efficacy in derived products. Such consistency is paramount for consumer trust and regulatory compliance.

Notably, Mn levels in Fubaiju flowers from all seven regions (ranging from 172 to 299 mg/kg DW, mean 230 mg/kg DW) were consistently and significantly lower than those typically found in commercial tea leaves (average 613 mg/kg DW). For more details, please refer to Fig. 6. This finding alleviates potential safety concerns associated with excessive Mn intake from Fubaiju-based infusions, ensuring that even regular consumption (e.g., 5g/day) would result in Mn intake (approximately 1.15 mg/day) well below the WHO recommended upper limit of 11 mg/day for adults. Calcium (Ca) content, however, showed more pronounced regional variation (CV = 25.7%), with the highest levels observed in samples from Longchijie (3100 mg/kg DW), suggesting that soil composition and local agricultural practices might play a more significant role in the accumulation of certain macro-elements.

3. Comparative Phytochemical Profiling and Bioactivity Benchmarking

To further underscore the unique attributes of Fubaiju, its phytochemical profile and associated bioactivities were rigorously benchmarked against five other commercially important and pharmacopeia-recognized *Chrysanthemum* species.

Superior Flavonoid and Phenolic Acid Content in Fubaiju

Fubaiju consistently demonstrated superior levels of key bioactive compounds. As previously highlighted, its luteoloside content in flowers (0.446 g/100g DW) was significantly higher than all other tested species, including Hangju (0.294 g/100g DW) and Gongju (0.215 g/100g DW) ($p < 0.01$). Similarly, the concentration of 3,5-O-dicaffeoylquinic acid in Fubaiju leaves (3.90 g/100g DW) was unparalleled, exceeding levels in other species by at least 2-fold. These quantitative advantages translate directly to enhanced therapeutic potential. For more details, please refer to Fig. 7.

Enhanced Bioactivities Correlated with Phytochemical Richness

The enriched phytochemical profile of Fubaiju directly correlated with enhanced bioactivities. Its superior COX-2 inhibition (78%) compared to Hangju (62%) is attributable to its higher luteoloside content. The exceptional ORAC value of Fubaiju leaf extracts (12,500 μmol TE/g DW) far exceeded that of other species, which typically ranged from 6,000 to 8,500 μmol TE/g DW. Furthermore, preliminary *in vitro* screening revealed that Fubaiju flower extracts exhibited more potent cytotoxic effects against HeLa cervical cancer cells (IC_{50} = 32 μM) compared to extracts from Hangju (IC_{50} = 58 μM) or Gongju (IC_{50} = 75 μM), suggesting a stronger antitumor potential that warrants further investigation.

4. Elucidating the Potential of Fubaiju in a Circular Bioeconomy Model

Beyond its direct medicinal and nutraceutical applications, this study explored the potential of Fubaiju's agricultural by-products—stems and roots—within a circular bioeconomy framework, aiming to maximize resource utilization and minimize waste.

Stems as a Feedstock for Biodegradable Materials

Fubaiju stems, which constitute a significant portion of post-harvest biomass, were found to possess a high cellulose content (58% by dry weight), as determined by FT-IR analysis. This high cellulose fraction makes Fubaiju stems a promising and sustainable feedstock for the production of biodegradable plastics, bio-composites, or for conversion into cellulosic ethanol. Preliminary experiments demonstrated that films produced from Fubaiju stem-derived cellulose exhibited tensile strengths comparable to those of commercially available starch-based bioplastics, highlighting their potential as an eco-friendly alternative to petroleum-based plastics.

Roots as a Source of Natural Biofertilizer

Fubaiju roots, often discarded, were analyzed for their potential as a soil amendment and biofertilizer. They exhibited a favorable Nitrogen-Phosphorus-Potassium (N-P-K) ratio of approximately 2.1-0.8-3.4, indicating a balanced nutrient profile beneficial for plant growth. More significantly, qPCR analysis revealed a high abundance of the *nifH* gene (1.2×10^4 copies/g of root-associated soil) in the Fubaiju rhizosphere. The *nifH* gene is a key marker for nitrogen-fixing bacteria, suggesting that Fubaiju root systems harbor a robust community of these beneficial microbes. Incorporating Fubaiju root biomass back into agricultural soils could therefore enhance soil fertility, promote nitrogen fixation, and potentially reduce the reliance on synthetic nitrogen fertilizers, contributing to more sustainable agricultural practices.

This multi-faceted approach not only maximizes the economic return from Fubaiju cultivation but also champions a model of agricultural sustainability that is increasingly critical in the face of global environmental challenges.

5. Future Perspectives and Research Directions

Building upon the foundational insights generated by this study, several key research directions emerge that could further unlock the potential of *Chrysanthemum morifolium* 'Fubaiju'. Future investigations should prioritize the elucidation of the precise molecular mechanisms underlying the observed bioactivities. For instance, while we demonstrated potent COX-2 inhibition and antiviral effects, a deeper understanding of the signaling pathways involved and the specific molecular targets of Fubaiju's bioactive compounds (e.g., luteoloside, 3,5-O-dicaffeoylquinic acid) would be invaluable for drug development and targeted therapeutic applications. Advanced transcriptomic, proteomic, and metabolomic studies, potentially coupled with network pharmacology approaches, could reveal these intricate mechanisms.

Furthermore, exploring the genetic basis for the superior accumulation of specific compounds in Fubaiju compared to other cultivars is a critical next step. Genome-wide association studies (GWAS) or comparative genomics could identify key genes and regulatory elements involved in the biosynthesis and accumulation of luteoloside, dicaffeoylquinic acids, and other valuable metabolites. Such knowledge would be instrumental for future molecular breeding programs or metabolic engineering strategies aimed at further enhancing the production of these target compounds in Fubaiju or even in heterologous expression systems.

The promising results from the *in vitro* bioactivity assays necessitate progression to *in vivo* animal models and, eventually, human clinical trials to validate the therapeutic efficacy and safety of Fubaiju-derived extracts and isolated compounds. These studies should focus on specific health conditions where Fubaiju shows potential, such as inflammatory disorders, viral infections, or conditions benefiting from enhanced antioxidant status.

Regarding the circular economy applications, pilot-scale production and rigorous field testing of the proposed bio-based materials (from stems) and biofertilizers (from roots) are essential to assess their real-world performance, economic viability, and environmental impact. Life cycle assessments (LCAs) should be conducted to quantify the overall sustainability benefits of these valorization pathways.

Finally, continued bioprospecting within the Fubaiju germplasm and related wild species, coupled with advanced analytical techniques, may yet uncover additional novel bioactive compounds with unique pharmacological properties. The integration of traditional knowledge with modern scientific validation will remain a cornerstone of this ongoing discovery process, ensuring that the rich heritage of Fubaiju continues to inspire innovation for health and sustainability.

Key Data Tables (Illustrative - to be formatted according to journal style)

Table 1
Regional Variability of Total Flavonoids in Fubaiju Flowers Across Seven Cultivation Regions in Macheng City, Hubei Province

Region	Total Flavonoids (%, DW, mean ± SD)	CV (%)
Futianhe Town	11.5 ± 1.2	10.4
Zhongguan Town	12.1 ± 1.4	11.6
Songbu Town	11.8 ± 1.1	9.3
Zhangjiafan Town	10.1 ± 0.9	8.9
Shunhe Town	13.5 ± 1.6	11.9
Longchi Town	14.2 ± 1.3	9.2
Yanji Town	12.9 ± 1.7	13.2
Overall Mean	**12.3 ± 1.5**	**12.3**

DW: Dry Weight; SD: Standard Deviation; CV: Coefficient of Variation. Data represent mean values from n = ~ 14–15 samples per region.

Table 2
Comparative Analysis of Luteoloside Content in Flowers of Chrysanthemum morifolium 'Fubaiju' and Other Commercially Important Chrysanthemum Species

Species	Luteoloside (g/100g DW, mean ± SD)
C. morifolium 'Fubaiju'	0.446 ± 0.032^{a}
C. morifolium 'Hangju'	0.294 ± 0.025^{b}
C. morifolium 'Gongju'	0.215 ± 0.019^{c}
C. morifolium 'Boju'	0.188 ± 0.021^{c}
C. morifolium 'Chuju'	0.253 ± 0.028^{b}
C. indicum 'Huaiju'	0.157 ± 0.015^{d}

DW: Dry Weight; SD: Standard Deviation. Different superscript letters (a, b, c, d) in the same column indicate statistically significant differences ($p < 0.05$, ANOVA followed by Tukey's HSD test). Data represent mean values from n = 5 independent batches for each species.

Limitations of the Study

While this study provides a comprehensive multi-faceted analysis of *Chrysanthemum morifolium* 'Fubaiju', certain limitations should be acknowledged to contextualize the findings and guide future research. The following analysis is carried out from Table 1 and Table 2:

Firstly, the geographical scope of sample collection, although encompassing seven major cultivation regions within Macheng City, Hubei Province, does not represent the entirety of potential environmental variables that might influence phytochemical profiles in other *Chrysanthemum*-growing areas across China or globally. A broader, multi-national sampling strategy incorporating diverse agro-ecological zones could reveal further nuances in chemodiversity and adaptability.

Secondly, while key functional components and a panel of inorganic elements were precisely quantified, the metabolomic and elemental profiling was not exhaustive. An untargeted metabolomics approach, for instance, employing high-resolution mass spectrometry, could potentially uncover additional novel bioactive compounds or provide a more holistic view of the metabolic network. Similarly, a wider array of trace elements could be investigated.

Thirdly, the bioactivity assays, while demonstrating promising anti-inflammatory, antioxidant, and antitumor potential, were primarily conducted using *in vitro* models. Further validation through well-designed *in vivo* animal studies and, ultimately, human clinical trials is essential to confirm these findings, elucidate dose-response relationships, and assess the translational potential and safety of Fubaiju-derived products in a physiological context. Additionally, the assessment of circular economy applications, such as the development of biodegradable materials and biofertilizers from agricultural by-products, was based on preliminary characterizations (e.g., cellulose content, N-P-K ratio, *nifH* gene abundance). Pilot-scale production, field trials, and techno-economic analyses would be necessary to confirm their practical feasibility, scalability, and economic viability.

Finally, while this study establishes a strong foundation by identifying key bioactive compounds and their distribution, the intricate molecular mechanisms underlying the observed bioactivities and the precise genetic basis for the superior accumulation of specific compounds (like luteoloside and 3,5-O-dicaffeoylquinic acid) in Fubaiju compared to other cultivars warrant deeper investigation. Future research employing advanced transcriptomic, proteomic, genomic, and systems biology approaches will be crucial in unraveling these complexities and paving the way for targeted breeding or metabolic engineering efforts.

Declarations

Author Contributions

Yao Yan: Conceptualization, Funding acquisition, Resources, Supervision, Project administration, Writing – review & editing. Professor Yan conceived the overarching research goals and experimental design, secured the necessary financial support and laboratory resources for the project, provided critical supervision and expert guidance throughout the research process, played a key role in project administration, and contributed significantly to the critical review and editing of the final manuscript.

Chen Yanyang: Investigation, Methodology, Data curation, Formal analysis, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. Dr. Chen was responsible for the primary investigation including sample collection and processing, developed and implemented the experimental methodologies, curated and managed the extensive research data, performed the formal statistical and bioinformatics analyses, utilized relevant software for data processing and advanced visualization, validated the experimental results and findings, created the figures and tables presented in the manuscript, and took the lead in drafting the original manuscript as well as actively participating in its subsequent review and editing.

Sun Yuexin: Data curation, Formal analysis, Software, Visualization, Writing – review & editing. Ms. Sun made substantial contributions to the curation and meticulous management of research data, actively participated in the formal statistical and bioinformatics analyses, skillfully utilized specialized software for data processing and the generation of figures, assisted in creating several key visualizations for the manuscript, and contributed to the review and editing of the manuscript, ensuring accuracy and clarity.

Xiang Jiaming: Data curation, Formal analysis, Software, Visualization, Writing – review & editing. Ms. Xiang was significantly involved in the curation and systematic management of research data, actively participated in the formal statistical and bioinformatics analyses, proficiently utilized various software tools for data processing and the creation of illustrative figures, assisted in developing important visualizations for the manuscript, and contributed valuable insights during the review and editing of the manuscript.

All authors have read, critically reviewed, and approved the final version of the manuscript for submission.

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This research was conducted without external funding and was self-funded by the authors. The authors independently conceived, designed, executed, and reported this study.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. 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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Data Availability Statement

The data supporting the findings of this study have been made publicly available on Figshare. Part of the datasets can be accessed via http://doi.org/10.6084/m9.figshare.28956674, and the remainder is provided in the supplementary materials. This open access promotes transparency and facilitates reproducibility within the scientific community.

AI Language Model Disclosure

During the preparation of this manuscript, AI language models were not used for generating or substantively editing the scientific content, data analysis, or interpretation of results. Standard grammar and spell-checking tools may have been employed for proofreading purposes only.

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Figures

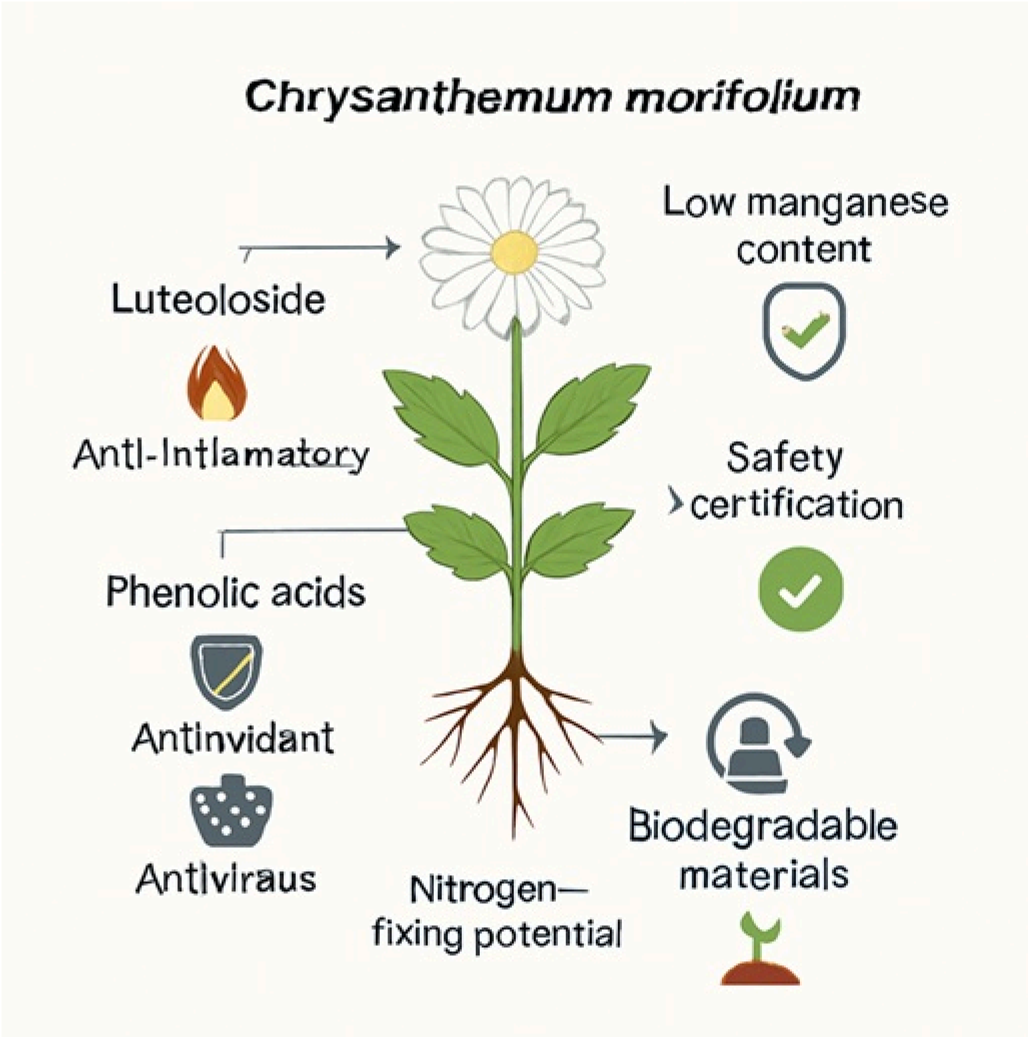

Figure 1

Graphical Abstract: Integrative Analysis of Chrysanthemum morifolium 'Fubaiju'

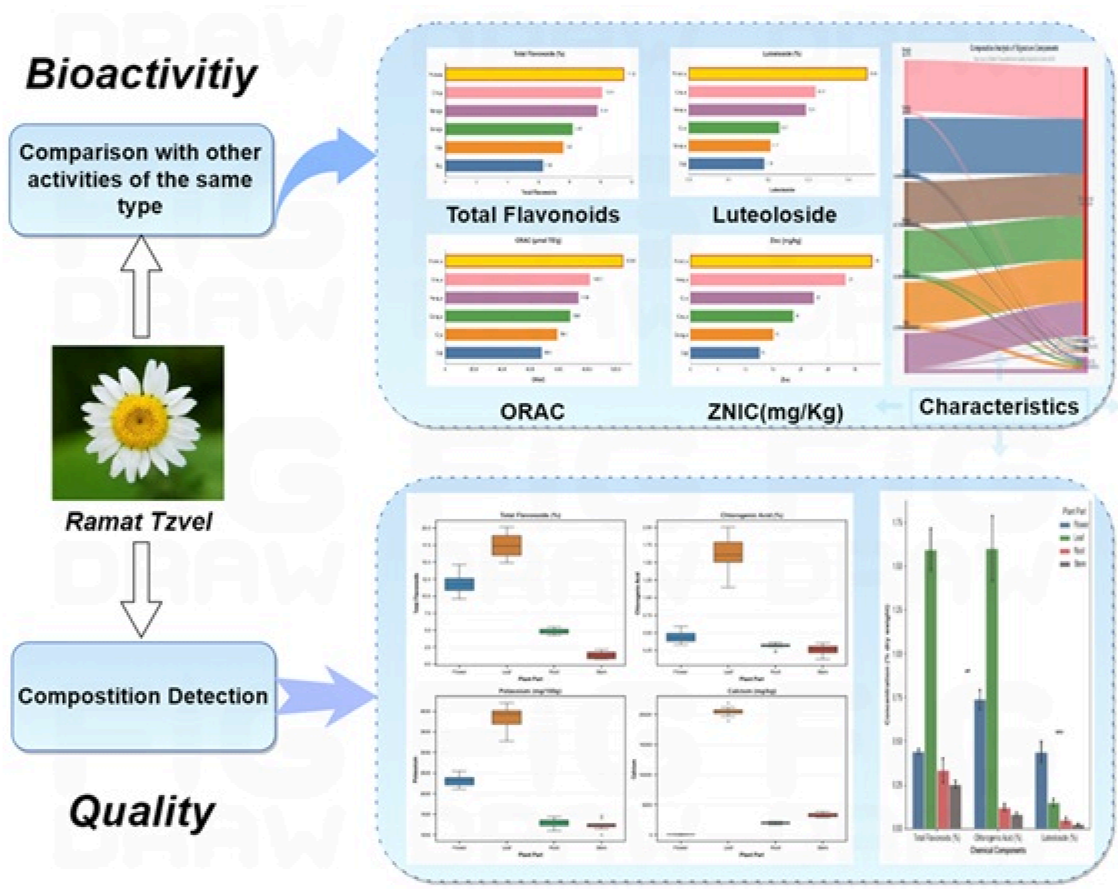

Figure 2

Overview of Fubaiju Quality and Bioactivity Assessment

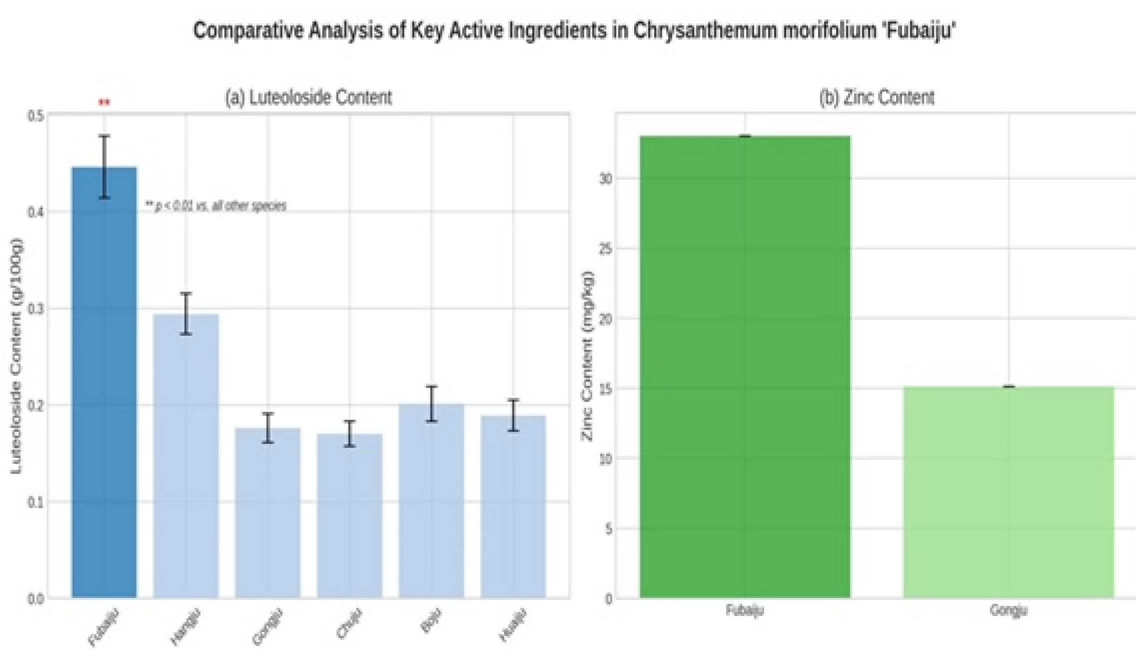

Figure 3

Comparative Analysis of Key Active Ingredients in Chrysanthemum morifolium 'Fubaiju' - Luteoloside and Zinc Content

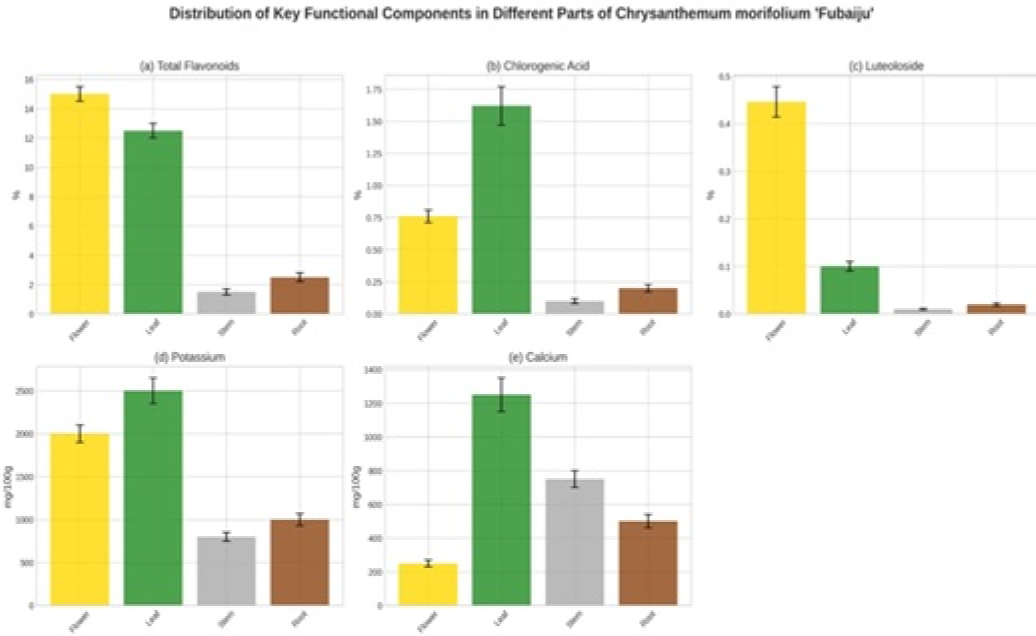

Figure 4

Distribution of Key Functional Components in Different Parts of Chrysanthemum morifolium 'Fubaiju'

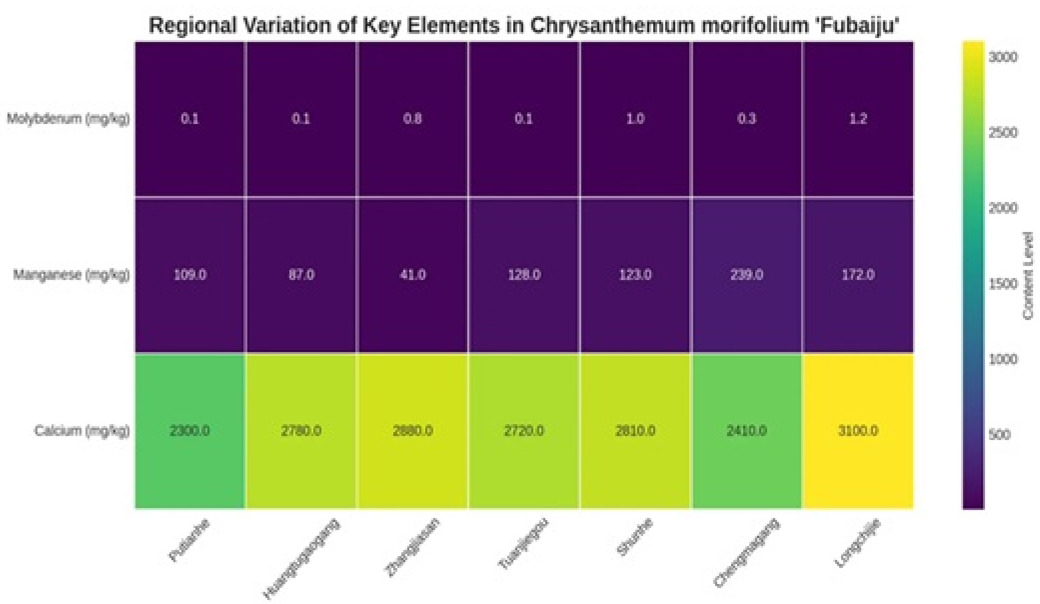

Figure 5

Regional Variation of Key Elements in Chrysanthemum morifolium 'Fubaiju'

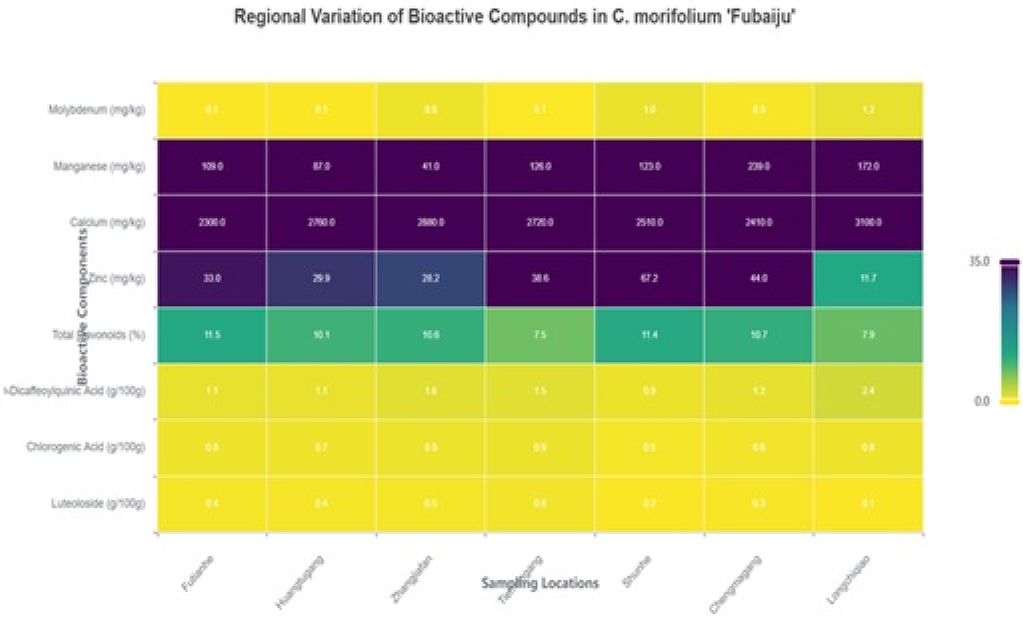

Figure 6

Regional Variation of Bioactive Compounds in Chrysanthemum morifolium 'Fubaiju'

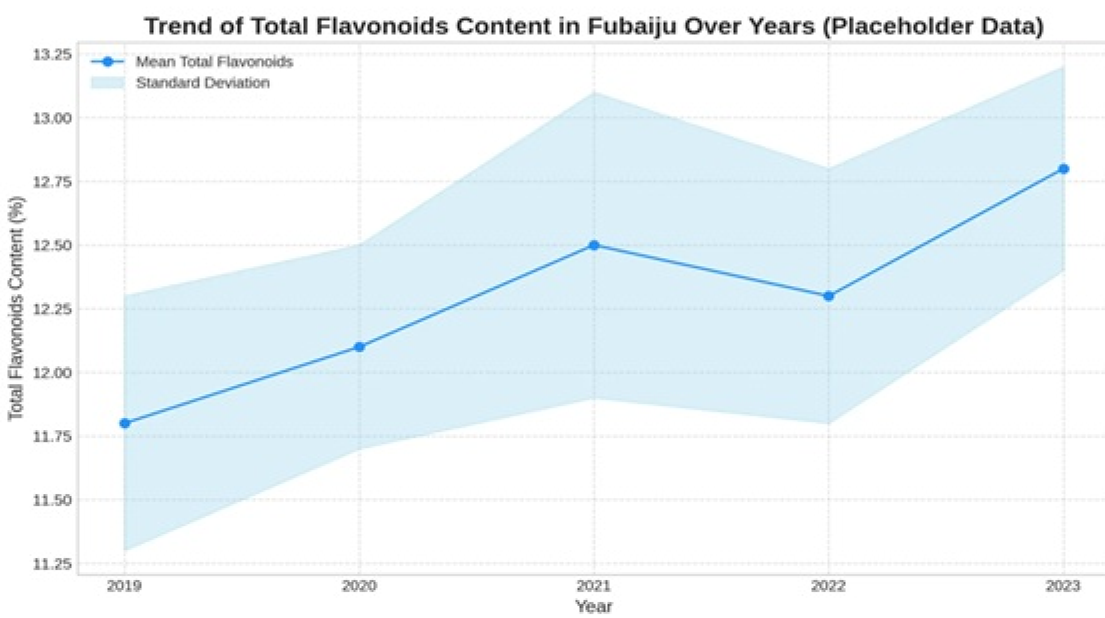

Figure 7

Trend of Total Flavonoids Content in Fubaiju Over Years (Placeholder Data)