



Semiconductor SERS Platform Enables Rapid Discrimination of Structurally Similar Flavonoids in Multicomponent Systems

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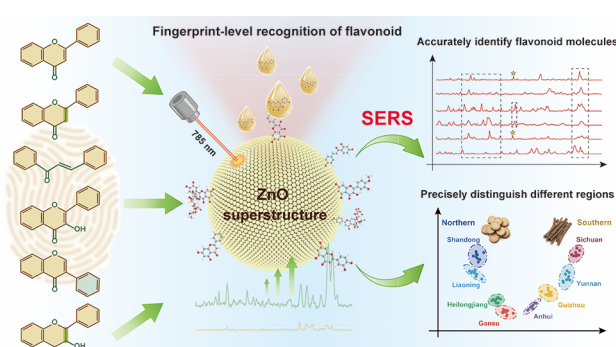
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Abstract: Rapid and reliable discrimination of structurally similar small molecules in complex systems remains a significant challenge for chemical sensing, particularly when subtle structural variations lead to highly overlapping spectral features. Herein, we develop a semiconductor surface-enhanced Raman scattering (SERS) platform based on ZnO superstructures for the reliable discrimination of structurally similar flavonoids in both single-component and multicomponent systems. The ZnO-based SERS platform enables the generation of molecule-specific spectral fingerprints, with characteristic variations in both skeletal vibration and functional group regions, allowing effective differentiation of closely related flavonoids. By integrating multivariate statistical analysis, robust classification, and discrimination of flavonoids with highly similar structures were achieved, including in mixed-component systems. The practical applicability of the platform is further validated using real plant-derived samples, where distinct spectral signatures enable effective discrimination of samples with different compositional profiles, thereby demonstrating its capability for botanical origin authentication. Notably, the proposed approach allows rapid analysis with minimal sample pretreatment and without the need for chromatographic separation. These results demonstrate that ZnO-based semiconductor SERS sensing provides a robust and scalable strategy for the detection and discrimination of structurally similar flavonoids in complex systems, highlighting its practical applicability for quality control and authenticity assessment in food and plant-derived materials.



Keywords: SERS; ZnO superstructures; flavonoids; multicomponent analysis; semiconductor sensing

1. Introduction

Rapid and reliable discrimination of structurally similar small molecules in complex systems remains a significant challenge in chemical sensing, particularly when subtle variations in molecular structure led to highly overlapping spectral features [1,2]. Such challenges are frequently encountered in plant-derived systems, where structurally related compounds coexist and exhibit minor differences in functional groups and substitution patterns [3,4]. Flavonoids, which are widely distributed in plant-derived systems and are known for their antioxidant and bioactive properties, are a representative class of such compounds, characterized by a common C6–C3–C6 framework with diverse substitution patterns and oxidation states [5–7] (Scheme 1a). These structural variations result in highly similar molecular backbones but subtle differences in functional groups, making their rapid identification and discrimination in complex matrices particularly challenging. Due to their structural diversity



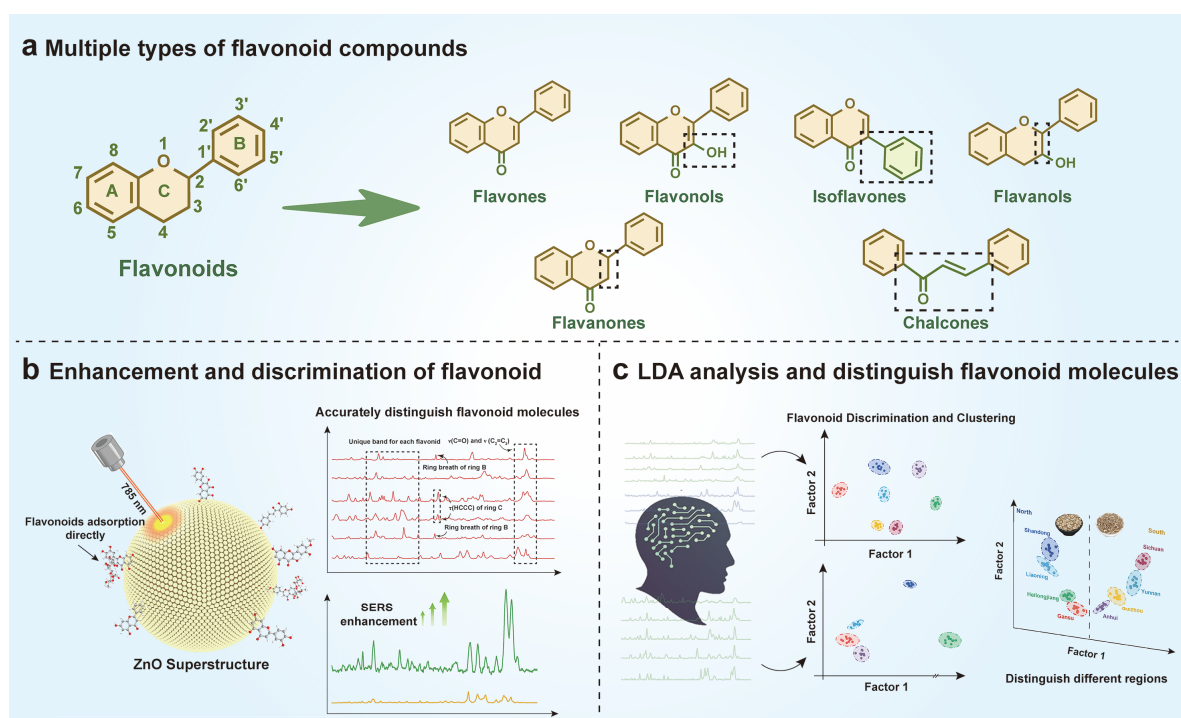
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and abundance, flavonoids are often used as characteristic markers for compositional evaluation of plant-derived materials. Therefore, developing effective analytical strategies for distinguishing structurally similar flavonoids is of considerable importance for compositional analysis and quality evaluation of plant-derived materials.

Surface-enhanced Raman scattering (SERS) provides molecular fingerprint specificity, rapid detection, minimal sample pretreatment, and low sample consumption, making it a promising tool for chemical sensing applications [8–17]. While noble-metal substrates can deliver high sensitivity, their practical use is often limited by cost, oxidation instability, and the need for surface modification [18–21]. In contrast, semiconductor-based SERS substrates offer improved chemical stability and can facilitate molecule-specific interactions through charge-transfer mechanisms [22–24]. In particular, ZnO is capable of interacting with hydroxyl-rich flavonoids via surface Zn^{2+} sites and hydroxyl groups, enabling adsorption-dependent signal modulation and enhancing sensitivity to subtle structural differences [25]. These interactions further modulate charge-transfer processes between ZnO and adsorbed molecules, providing a basis for selective enhancement of vibrational modes.

In this work, we develop a ZnO superstructure-based SERS platform for the discrimination of structurally similar flavonoids (Scheme 1b). The platform enables the generation of distinct spectral fingerprints while preserving intrinsic molecular vibrational features. By integrating chemometric analysis, reliable classification of flavonoids is achieved in both single-component and multicomponent systems. To demonstrate practical applicability, Isatis Root (Banlangen), a plant-derived material with known compositional variability, was selected as a representative system (Scheme 1c). Notably, northern and southern Isatis Root in China originate from different botanical sources and exhibit distinct chemical profiles, particularly in flavonoid composition [26]. These differences provide a suitable model system for evaluating the capability of the proposed SERS platform in discriminating complex samples with subtle compositional variations [27,28]. This approach provides a rapid and practical strategy for the analysis of structurally similar compounds in complex systems.



Scheme 1. Conceptual illustration of SERS-based flavonoid discrimination and application workflow. (a) Representative structural classes of flavonoids. (b) Schematic illustration of SERS-based molecular fingerprinting of flavonoids on ZnO superstructure substrates. (c) Taking northern and southern Isatis Roots as examples, distinguished and classified flavonoids from different plant sources.

2. Experimental Section

2.1. Chemicals

Zinc acetate dihydrate, diethylene glycol, absolute ethanol, chromatographic-grade methanol, luteolin, chrysin, rutin, quercetin, and kaempferol were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Isorhamnetin, naringenin, liquiritigenin, L-epicatechin, genistein, isoliquiritigenin, linarin, and neohesperidin were obtained from Rhawn Chemical Technology Co., Ltd. (Shanghai, China). Traditional Chinese medicinal samples

were collected from Shandong, Gansu, Liaoning, Heilongjiang, Sichuan, Yunnan, Guizhou, and Anhui provinces, China. All solutions were prepared using analytical-grade reagents and Milli-Q water.

2.2. Characterization Methods

Scanning electron microscopy (SEM) images were acquired using a JSM-7500F field-emission scanning electron microscope (JEOL Ltd., Tokyo, Japan). X-ray diffraction (XRD) patterns were recorded on a Shimadzu XRD-6100 diffractometer with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) at room temperature (Shimadzu Corporation, Kyoto, Japan). Raman and SERS spectra were measured using a Raman spectrometer (Zolix Finder Insight FI785-Pro) equipped with a 785 nm excitation laser (Zolix Instruments Co., Ltd., Beijing, China). The instrument was calibrated using the 520.7 cm^{-1} vibrational band of a silicon wafer.

2.3. Synthesis of ZnO Superstructures

ZnO superstructures were synthesized via a solvothermal method. Briefly, 0.01 mol of zinc acetate dihydrate was dissolved in 100 mL of diethylene glycol and heated slowly to 160°C under magnetic stirring for 1 h, forming a turbid colloidal suspension. After cooling to room temperature, the suspension was centrifuged, and the supernatant was collected as the seed solution. Subsequently, another 0.01 mol of zinc acetate dihydrate was dissolved in 100 mL of diethylene glycol and heated to 130°C . Then, 0.3 mL of the seed solution was introduced into the hot solution, and the temperature was rapidly increased to 160°C and maintained for 1 h to obtain spherical ZnO particles. The resulting nanoparticles were collected by centrifugation, washed three times with distilled water and absolute ethanol, and finally dried in a vacuum oven at 60°C for 12 h before use.

2.4. Preparation of Chinese Medicine Samples and SERS Measurements

Chinese medicinal samples from different geographical origins were ground into fine powders using a grinder. Approximately 0.5 g of powder was dispersed in chromatographic-grade methanol and ultrasonically extracted for 90 min. The organic phase was collected and concentrated to dryness under reduced pressure using a rotary evaporator. Prior to LC–MS/MS analysis, the extract was re-dissolved in 10 mL of methanol and filtered through a $0.45 \text{ }\mu\text{m}$ nylon membrane. For SERS measurements, 10 μL of the crude extract was dropped onto a ZnO superstructure substrate dispersed in ethanol and allowed to dry naturally. The flavonoid signals were directly recorded using a 785 nm Raman spectrometer with a laser power of 20 mW and an acquisition time of 3 s. The obtained spectra were baseline-corrected and smoothed using LabSpec 5 software (Version) prior to further analysis.

2.5. LDA Analysis of SERS Spectra

Linear discriminant analysis (LDA) was performed using the SERS spectra of crude Chinese medicinal samples extracts collected over the range of $200\text{--}2000 \text{ cm}^{-1}$. All spectra were preprocessed by baseline correction and smoothing before analysis in SPSS Statistics 25.0. The processed data were subsequently visualized and further analyzed using Origin 2023 to generate two-dimensional LDA score plots, enabling effective discrimination among different flavonoids and TCM samples from various geographical origins.

3. Result and Discussion

3.1. Analytical Performance of ZnO Superstructure-Based SERS Platform

The ZnO superstructure substrate was designed and evaluated with a focus on its analytical suitability for SERS-based flavonoid analysis. The near-field scattering efficiency (Q_{NF}) of spherical ZnO particles was theoretically calculated based on Mie theory (Figure 1a,b), indicating that particles with a diameter around 400 nm led to enhanced local electric-fields under 785 nm excitation. This size-dependent optical response provides a rational basis for optimizing SERS performance. Based on this consideration, ZnO superstructures were synthesized via a polyol-mediated precipitation method, forming uniform spherical architectures assembled from nanoscale primary particles [8]. As shown in Figure 1c,d, the structures exhibit good morphological uniformity with an average diameter of approximately $492.62 \pm 2.02 \text{ nm}$, which is beneficial for achieving reproducible SERS responses. X-ray diffraction analysis (Figure 1e) confirms the formation of phase-pure hexagonal wurtzite ZnO, ensuring structural reliability of the sensing substrate.

The SERS performance of the ZnO superstructures was evaluated using rutin as a model analyte (Figure 1f). The obtained SERS spectrum shows peak positions highly consistent with its intrinsic Raman spectrum, indicating that the platform preserves characteristic molecular vibrational features. In contrast, Ag substrate yields weak and

limited signals due to the lack of adsorption sites for rutin molecules. The superior performance of ZnO is attributed to its ability to facilitate direct interaction with hydroxyl-rich flavonoid molecules, enabling efficient signal transduction without additional surface modification. To further evaluate the generality of the platform, the SERS spectra of multiple representative flavonoids were collected and compared with their intrinsic Raman spectra (Figures S1–S7). The results show that ZnO superstructures not only provide significant signal enhancement but also largely preserve intrinsic vibrational features, demonstrating their suitability for fingerprint-based discrimination of structurally similar compounds. To evaluate the reproducibility of the ZnO superstructure-based SERS platform, repeated measurements were performed for representative flavonoid molecules, and the corresponding relative standard deviation (RSD) values were calculated (Figure S8). The obtained RSD values ranged from 2.76% to 4.86% among the 13 investigated flavonoids, demonstrating high spectral reproducibility of the substrate. The low variation among repeated measurements can be attributed to the uniform morphology of ZnO superstructures and the stable molecule substrate interactions, confirming their suitability for reliable flavonoid sensing and discrimination.

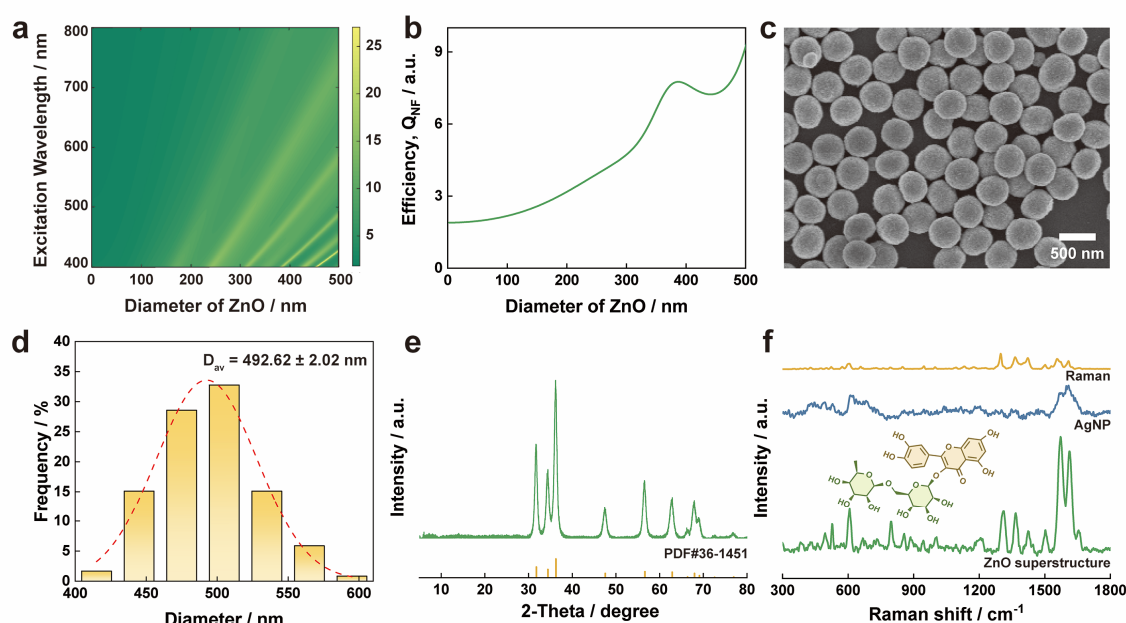


Figure 1. Structural characterization and SERS performance of ZnO superstructures. (a) Calculated near-field scattering efficiency of ZnO microspheres as a function of incident wavelength and particle diameter. (b) Near-field scattering efficiency of ZnO particles as a function of particle diameter under 785 nm excitation. (c) SEM image of ZnO superstructures. (d) Particle size distribution of ZnO superstructures. (e) XRD pattern of ZnO superstructures. (f) SERS spectra of rutin obtained on ZnO superstructures (green) and Ag nanoparticle (AgNP) substrates (blue), together with the intrinsic Raman spectrum of rutin powder (yellow).

The enhanced SERS activity of ZnO superstructures can be attributed to the synergistic effect of adsorption-dependent interactions, charge-transfer (CT) enhancement, and size-induced electric field enhancement. Flavonoid molecules containing hydroxyl and carbonyl groups can strongly interact with surface Zn^{2+} sites and hydroxyl groups, leading to favorable adsorption configurations and enhanced electronic coupling. These interactions facilitate charge-transfer transitions between ZnO and the adsorbed molecules, which selectively enhance specific vibrational modes. In addition, the optimized particle size contributes to Mie resonance-induced local field enhancement, further improving the overall SERS response. The synergistic effect of these factors enables sensitive and discriminative detection of structurally similar flavonoids.

3.2. Spectral Fingerprints and Discrimination of Individual Flavonoids

To evaluate the capability of the ZnO-based SERS platform for discriminating structurally similar molecules, representative flavonoids with different substitution patterns and structural types were systematically investigated (Figure 2). Despite sharing a common C6–C3–C6 backbone, variations in the type, position, and number of substituents on the A, B, and C rings underpin the structural diversity of flavonoid molecules. According to their structural characteristics, six representative flavonoids from distinct structural subclasses, including chrysin

(flavone), quercetin (flavonol), naringenin (flavanone), epicatechin (flavanol), genistein (isoflavone), and isoliquiritigenin (chalcone), were selected to be systematically investigated (Figure 2). To systematically evaluate the discrimination capability of the proposed SERS platform, structurally similar flavonoids within the same subclass were first selected to assess its sensitivity to subtle molecular variations, followed by representative flavonoids from different subclasses to evaluate its ability to distinguish broader structural differences.

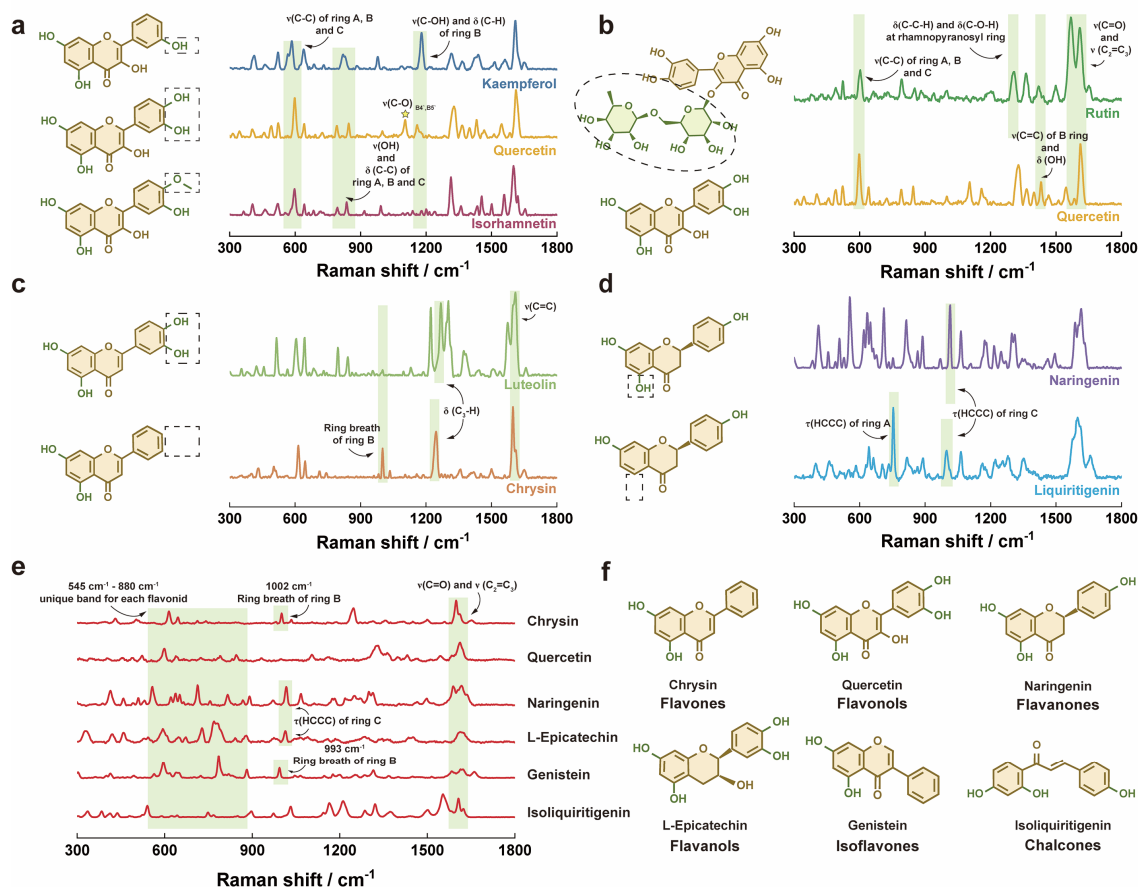


Figure 2. SERS spectral fingerprints of representative flavonoids. (a) Chemical structures and SERS spectra of three flavonols: kaempferol, quercetin, and isorhamnetin. (b) Chemical structures and SERS spectra of rutin and quercetin. (c) Chemical structures and SERS spectra of luteolin and chrysin. (d) Chemical structures and SERS spectra of naringenin and liquiritigenin. (e) SERS spectra of six representative flavonoid subclasses: chrysin (flavone), quercetin (flavonol), naringenin (flavanone), epicatechin (flavanol), genistein (isoflavone), and isoliquiritigenin (chalcone). (f) Corresponding chemical structures of the six flavonoids shown in (e).

Kaempferol, quercetin, and isorhamnetin are representative flavonols (Figure 2a). Specifically, kaempferol contains a single hydroxyl group at the 5' position of the B ring; quercetin possesses two hydroxyl groups at the 4' and 5' positions; and isorhamnetin is derived from quercetin via substitution of the 4'-hydroxyl group with a methoxy group. Although these structural differences are subtle, their SERS spectra exhibit clear and reproducible distinctions. For kaempferol, the C–C stretching vibrations of the A, B, and C rings split into two characteristic peaks at 567 and 581 cm^{-1} whereas the corresponding vibrations of quercetin and isorhamnetin appear as a single peak at 595 cm^{-1} . Quercetin and isorhamnetin both exhibit two peaks at 787 and 848 cm^{-1} , attributed to ring vibrations involving O–H and C–C modes, while kaempferol shows only one peak at 820 cm^{-1} [29]. In addition, kaempferol presents a strong peak at 1178 cm^{-1} assigned to C–OH stretching and C–H bending vibrations of the B ring, whereas quercetin displays two overlapping peaks at 1161 and 1176 cm^{-1} , and no prominent peak is observed for isorhamnetin in this region. Notably, quercetin exhibits a unique peak at 1102 cm^{-1} , which is absent in kaempferol and isorhamnetin, providing a useful diagnostic feature associated with the presence of adjacent hydroxyl groups on the B ring.

Quercetin and rutin belong to different subclasses of flavonoids. Quercetin is a flavonol, whereas rutin is a flavonol glycoside formed by linking the 3-hydroxyl group of the aglycone to a rutinose moiety via a glycosidic bond. Compared with quercetin, rutin contains additional hydroxyl groups and exhibits a more complex molecular

structure (Figure 2b). Their SERS spectra are highly similar below 900 cm^{-1} ; however, at approximately 600 cm^{-1} , the C–C stretching vibration shows markedly higher intensity for quercetin than that for rutin. Rutin exhibits a broad band spanning $1390\text{--}1490\text{ cm}^{-1}$ with a sharp peak at 1419 cm^{-1} , corresponding to the peak at 1430 cm^{-1} for quercetin, both assigned to C=C stretching and O–H bending vibrations of the B ring [30]. Furthermore, rutin displays two peaks of comparable intensity at 1567 and 1607 cm^{-1} , which are attributed to C=O stretching and $\text{C}_2=\text{C}_3$ stretching vibrations, whereas quercetin shows only a single peak at 1612 cm^{-1} . These differences provide effective spectral markers for distinguishing glycosylated and non-glycosylated flavonoids.

Luteolin and chrysin are both flavones, differing from flavanols by the absence of a hydroxyl group at the 3' position. The key structural difference lies in the presence of two adjacent hydroxyl groups on the B ring of luteolin, whereas chrysin lacks substituents on its B ring (Figure 2c). In the SERS spectra, chrysin exhibits a distinct peak at 1002 cm^{-1} that is absent in luteolin, attributable to an undisturbed ring-breathing vibration enabled by the unsubstituted B ring [31–33]. In the range of $1200\text{--}1320\text{ cm}^{-1}$, chrysin displays a single strong peak at 1244 cm^{-1} assigned to $\text{C}_3\text{--H}$ bending vibration, while luteolin shows three distinct peaks, among which the peak at 1269 cm^{-1} shares the same vibrational assignment as the 1244 cm^{-1} peak of chrysin. Additionally, luteolin presents two characteristic peaks at 1574 and 1609 cm^{-1} , whereas chrysin exhibits a single, relatively broad peak at 1598 cm^{-1} corresponding to C=C stretching vibration. These features enable reliable differentiation between substituted and unsubstituted B-ring structures.

Naringenin and liquiritigenin are flavanones characterized by fully saturated C-ring positions at C_2 and C_3 . Compared with liquiritigenin (Figure 2d), naringenin contains an additional hydroxyl group at the 5 position of the A ring, which enhances its interaction with the ZnO surface and results in stronger SERS signals. In addition to intensity differences, distinct spectral features are observed: liquiritigenin exhibits a sharp peak at 752 cm^{-1} attributed to A-ring torsional vibration, while peaks associated C-ring torsional vibration appear at 1022 cm^{-1} for naringenin and 995 cm^{-1} for liquiritigenin, with the latter showing substantially lower intensity [34,35].

These spectral differences can be attributed to structure-dependent adsorption configurations and interfacial electronic interactions between flavonoids and the ZnO surface. Hydroxyl and carbonyl groups in flavonoids can interact with surface Zn^{2+} sites and hydroxyl groups, leading to different adsorption geometries. Variations in adsorption configuration affect the degree of orbital overlap and electronic coupling, thereby modulating charge-transfer processes between ZnO and the adsorbed molecules. As a result, specific vibrational modes are selectively enhanced, and small peak shifts are observed in the SERS spectra. These differences further demonstrate the sensitivity of the ZnO-based SERS platform to subtle structural variations.

Accordingly, systematic spectral assignment and comparison were conducted for representative flavonoids across multiple subclasses. All flavonoids exhibit distinct fingerprint features, particularly within the $545\text{--}880\text{ cm}^{-1}$ region (Figure 2e), which serves as a key spectral window for classification [32]. For molecules lacking B-ring substituents, such as chrysin and genistein, display characteristic B-ring breathing vibrations near 1000 cm^{-1} , whereas flavanones and flavanols with saturated C rings show characteristic torsional modes near 1015 cm^{-1} . In addition, variations in peak position, intensity, and line shape within the $1580\text{--}1630\text{ cm}^{-1}$ region, corresponding to C=O and $\text{C}_2=\text{C}_3$ stretching vibrations, further contribute to molecular differentiation. These results collectively establish a set of diagnostic spectral features for flavonoid identification and provide a robust analytical basis for resolving structurally similar compounds. Importantly, the well-defined and reproducible spectral differences observed here lay the foundation for extending this approach to the analysis of multicomponent systems, as discussed in the following section.

3.3. Discrimination of Flavonoids in Multicomponent Systems

The capability of the ZnO-based SERS platform for resolving structurally similar flavonoids was further evaluated in multicomponent systems. Mixed detection of structurally related molecules provides a direct assessment of analytical applicability and further validates the diagnostic spectral features identified for individual compounds. To this end, representative flavonoid subclasses, including flavones and flavanols, were selected for binary and multicomponent mixture analysis.

For flavones, binary mixtures of chrysin and luteolin were prepared at different ratios (Figure S9a–c). As the proportion of chrysin decreased, the characteristic peak at 1002 cm^{-1} , assigned to B-ring breathing vibration, shows a pronounced reduction in intensity. In the $1150\text{--}1350\text{ cm}^{-1}$ region, the gradual shift of the in-plane C–H bending vibration from 1244 cm^{-1} toward 1269 cm^{-1} was observed with increasing luteolin content, consistent with the spectral features of the pure components (Figure S9d). These results demonstrate that both intensity variation and peak position shifts can serve as effective indicators for resolving structurally similar flavonoids in binary systems.

Similar mixture experiments were conducted for two flavonols, including kaempferol and quercetin (Figure 3a–c). Samples 1–4 corresponded to kaempferol/quercetin molar ratios of 4:1, 3:2, 2:3, and 1:4, respectively. As shown in Figure 3d, in the 555–620 cm^{-1} region, when quercetin predominated in sample 4, the C–C stretching vibration of the A, B, and C rings was dominated by a strong peak at 597 cm^{-1} , with the 585 cm^{-1} peak appearing as a shoulder. As the quercetin content decreased, this dominant peak gradually shifted toward 585 cm^{-1} , while the characteristic kaempferol peak at 567 cm^{-1} increased markedly with increasing kaempferol proportion. Meanwhile, in the 750–900 cm^{-1} region, the evolution from a single peak (820 cm^{-1} , kaempferol) to two distinct peaks (787 and 848 cm^{-1} , quercetin) further confirms the composition-dependent spectral response. In addition, the intensity of the kaempferol-specific peak at 1178 cm^{-1} decreased systematically with decreasing kaempferol proportion. These results indicate that the SERS spectra provide consistent and composition-sensitive features for distinguishing flavonol mixtures.

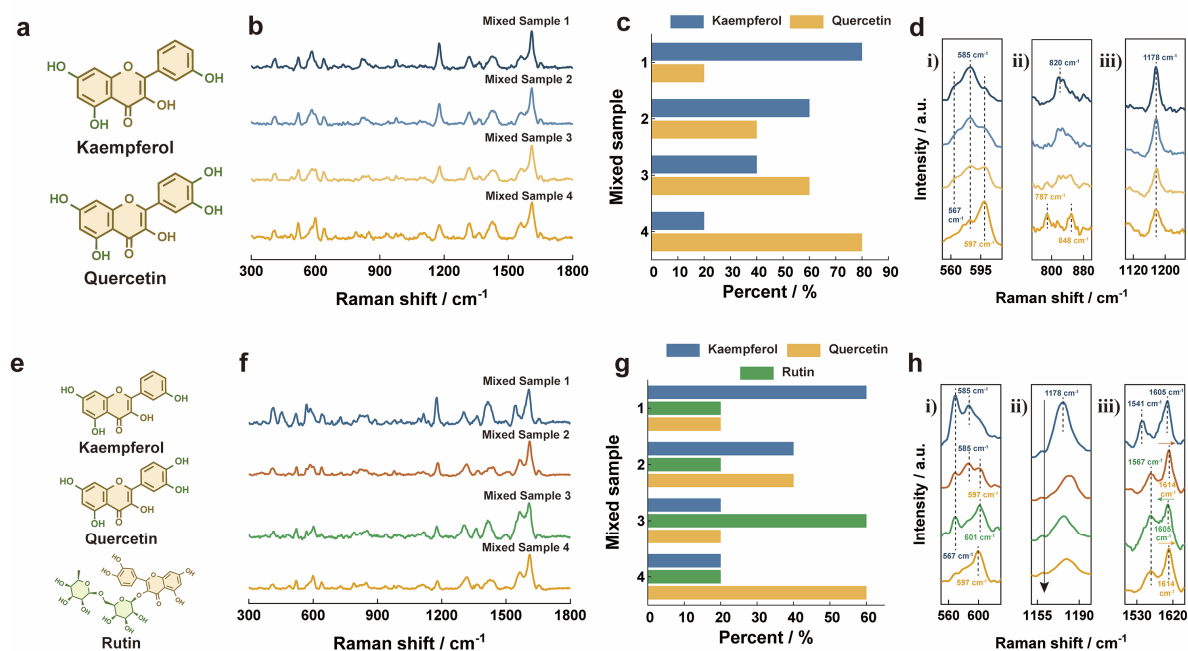


Figure 3. SERS analysis of binary and ternary flavonoid mixtures. (a–d) Binary mixtures of kaempferol and quercetin: (a) chemical structures; (b) SERS spectra; (c) composition ratios; (d) expanded spectral regions (555–620, 750–900, and 1110–1250 cm^{-1}). (e–h) Ternary mixtures of kaempferol, quercetin, and rutin: (e) chemical structures; (f) SERS spectra; (g) composition ratios; (h) expanded spectral regions (550–630, 1150–1200, and 1500–1650 cm^{-1}). Binary mixtures were prepared at molar ratios of 4:1, 3:2, 2:3, and 1:4, while ternary mixtures were prepared at molar ratios of 3:1:1, 2:1:2, 1:3:1, and 1:1:3. For all binary and ternary mixtures, the total flavonoid concentration was fixed at 10^{-3} M, and the concentration of each component was calculated according to its stated molar fraction. For the binary ratios of 4:1, 3:2, 2:3, and 1:4, the corresponding component concentrations were 8.0/2.0, 6.0/4.0, 4.0/6.0, and $2.0/8.0 \times 10^{-4}$ M, respectively. For the ternary ratios of 3:1:1, 2:1:2, 1:3:1, and 1:1:3, the corresponding concentrations were 6.0/2.0/2.0, 4.0/2.0/4.0, 2.0/6.0/2.0, and $2.0/2.0/6.0 \times 10^{-4}$ M, respectively.

To further evaluate the discrimination of glycosylated and non-glycosylated species, mixtures of rutin and quercetin were analyzed (Figure S9a–c). With increasing quercetin content, the intensity of the C–C stretching vibration near 597 cm^{-1} increased correspondingly. In the 1380–1470 cm^{-1} region, the spectral profile of the mixtures gradually evolved from the broad band characteristic of rutin toward that of quercetin, reflecting differences in molecular structure and hydrogen-bonding interactions. In the high-wavenumber region, the peak near 1607 cm^{-1} gradually shifted toward 1612 cm^{-1} with increasing quercetin proportion, accompanied by an increase in intensity (Figure S10d). These composition-dependent spectral changes enable effective differentiation between glycoside and aglycone forms.

Furthermore, ternary mixture experiments involving kaempferol, quercetin, and rutin were performed to evaluate the performance of the platform in more complex systems (Figure 3e–g). Across all samples, the SERS spectra retained characteristic features of the individual components, particularly in the regions of 550–630 cm^{-1} and 1500–1650 cm^{-1} . The intensity variation of characteristic peaks, such as the kaempferol-specific band at 1178 cm^{-1} , closely followed the changes in composition, while minor peak shifts further reflected differences in molecular

interactions (Figure 3h). Collectively, these results demonstrate that the ZnO-based SERS platform provides composition-dependent and reproducible spectral responses, enabling reliable discrimination of structurally similar flavonoids in both binary and multicomponent systems without chromatographic separation.

3.4. Application to Real Samples: Authentication of *Isatis Root*

Isatis Root is one of the most widely used traditional Chinese medicinal materials and is rich in flavonoid bioactive components, including quercetin, liquiritigenin, and isorhamnetin [6,32]. Notably, northern *Isatis Root* (derived from *Isatis indigotica* Fort., Brassicaceae) and southern *Isatis Root* (derived from *Baphicacanthus cusia* (Nees) Bremek., Acanthaceae) differ significantly in botanical origin, chemical composition, and pharmacological activity [27,28,36,37]. Accurate discrimination between northern and southern *Isatis Root* is therefore important for compositional analysis and quality evaluation.

Flavonoid components were extracted from commercially available samples using ultrasonic extraction. LC–MS/MS analysis was first employed as a reference method (Figures 4a,b and S11–S13), confirming distinct compositional profiles between northern and southern *Isatis Root*. Northern samples were found to predominantly contain kaempferol, rutin, neohesperidin, linarin, liquiritigenin, quercetin, and isorhamnetin, whereas southern samples were enriched in quercetin, kaempferol, isorhamnetin, epicatechin, and luteolin [38,39]. These compositional differences provide a chemical basis for subsequent SERS-based discrimination. The ZnO superstructures were subsequently used as SERS substrates to analyze the extracted samples, and linear discriminant analysis (LDA) was applied for classification (Figure 4c,f). The results demonstrate that distinct SERS spectral signatures corresponding to different flavonoid compositions were observed, and LDA yielded well-separated clusters, demonstrating the capability of the method for reliable discrimination of samples with different botanical origins. To further elucidate the spectral variables responsible for sample discrimination, principal component loading analysis was performed as a variable contribution assessment (Figure S14). The loading plots of PC1 and PC2 illustrate the contribution of individual Raman shift variables to the corresponding principal components. Raman variables with higher absolute loading coefficients represent spectral regions that contribute more significantly to the differentiation of samples. Several characteristic Raman regions exhibited relatively large loading coefficients, suggesting that these vibrational features play important roles in describing the spectral differences among *Isatis Root* samples. These discriminative regions are mainly associated with molecular skeletal vibrations and functional group vibrations of flavonoid-related components.

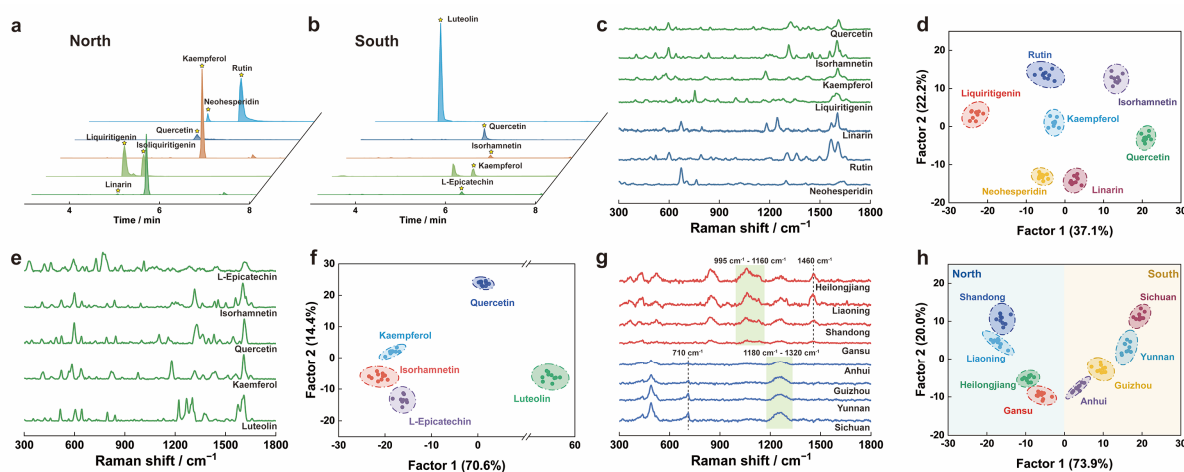


Figure 4. Application of the ZnO-based SERS platform for discrimination of plant-derived samples. (a,b) LC–MS/MS analysis of flavonoid composition in crude extracts of northern (Heilongjiang) and southern (Sichuan) *Isatis Root*. (c,d) Representative flavonoids in northern *Isatis Root*: (c) SERS spectra (10^{-3} M) of quercetin, isorhamnetin, kaempferol, liquiritigenin, linarin, rutin, and neohesperidin; (d) corresponding LDA score plot. (e,f) Representative flavonoids in southern *Isatis Root*: (e) SERS spectra (10^{-3} M) of epicatechin, isorhamnetin, quercetin, kaempferol, and luteolin; (f) corresponding LDA score plot. (g,h) Analysis of crude extracts from multiple regions: (g) SERS spectra of ultrasonic extracts from northern (red) and southern (blue) *Isatis Root* samples; (h) LDA score plot of samples collected from eight different regions.

To further evaluate practical applicability, *Isatis Root* samples collected from eight provinces across northern and southern China were analyzed (Figure 4g–h). Although the overall spectral profiles are similar due to shared

flavonoid components, clear differences in characteristic peak intensities were observed, enabling discrimination of geographical origin (Figure 4g). Northern samples exhibit prominent peaks in the 995–1160 cm^{-1} region, associated with C-ring torsional vibrations in liquiritigenin, along with a distinct peak at 1460 cm^{-1} attributed to $\text{C}_3\text{--H}$ bending vibration of rutin. In contrast, southern samples display a characteristic peak at 710 cm^{-1} related to C–C stretching vibrations of rings A, B, and C in epicatechin, as well as a broad band in the 1180–1320 cm^{-1} region corresponding to $\text{C}_3\text{--H}$ bending vibration of luteolin. These spectral differences enabled effective discrimination of geographical origin.

The LDA results exhibit clear clustering of samples from different provinces, with all samples falling within their respective 95% confidence ellipses, and more importantly, northern and southern samples symmetrically separated on opposite sides of the zero axis (Figure 4h). Notably, this differentiation was achieved without chromatographic separation and with minimal sample pretreatment. These findings demonstrate that ZnO-based SERS combined with multivariate statistical analysis offers a powerful and reliable strategy for the classification and identification of traditional Chinese medicine samples, highlighting its potential for quality control and origin discrimination in complex plant-derived systems.

4. Conclusions

In conclusion, we developed a ZnO superstructure-based semiconductor SERS platform for the rapid discrimination of structurally similar flavonoids. The optimized ZnO architectures provide effective molecule substrate interactions and enhanced charge transfer processes, enabling the acquisition of characteristic Raman fingerprints with high spectral reproducibility. By combining SERS with multivariate statistical analysis, the proposed platform successfully differentiated individual flavonoids, multicomponent mixtures, and plant-derived samples with subtle compositional differences. Furthermore, the analysis of real Isatis Root samples demonstrates the potential of this strategy for rapid quality evaluation and authenticity assessment of complex botanical materials. This work highlights the capability of semiconductor SERS platforms for resolving challenging molecular discrimination problems beyond conventional sensitivity-oriented detection, providing a practical approach for chemical sensing and natural product analysis.

Supplementary Materials: The following supporting information can be downloaded at: <https://media.sciltp.com/articles/others/2608261533227573/MI-26060131-SM.pdf>. Figure S1: Comparison of the SERS spectra (green) and powder Raman spectra (yellow) of chrysin (a) and luteolin (b); Figure S2: Comparison of the SERS spectra (green) and powder Raman spectra (yellow) of kaempferol (a), quercetin (b) and isorhamnetin (c); Figure S3: Comparison of the SERS spectra (green) and powder Raman spectra (yellow) of naringenin (a) and liquiritigenin (b); Figure S4: Comparison of the SERS spectra (green) and powder Raman spectra (yellow) of neohesperidin (a) and linarin (b); Figure S5: Comparison of the SERS spectra (green) and powder Raman spectra (yellow) of isoliquiritigenin; Figure S6: Comparison of the SERS spectra (green) and powder Raman spectra (yellow) of L-epicatechin; Figure S7: Comparison of the SERS spectra (green) and powder Raman spectra (yellow) of genistein; Figure S8: Reproducibility evaluation of ZnO superstructure-based SERS measurements. (a–m) Repeated SERS spectra and corresponding RSD values of 13 representative flavonoids, including chrysin, luteolin, kaempferol, quercetin, isorhamnetin, naringenin, liquiritigenin, neohesperidin, linarin, rutin, isoliquiritigenin, L-epicatechin, and genistein; Figure S9: (a–d) Binary mixtures of chrysin and luteolin: (a) chemical structures; (b) SERS spectra (10^{-3} M); (c) composition ratios; (d) expanded spectral regions (950–1050, 1150–1350, and 1550–1650 cm^{-1}). Binary mixtures were prepared at molar ratios of 4:1, 3:2, 2:3, and 1:4, corresponding to individual concentrations of 8.0×10^{-4} and 2.0×10^{-4} M, 6.0×10^{-4} and 4.0×10^{-4} M, 4.0×10^{-4} and 6.0×10^{-4} M, and 2.0×10^{-4} and 8.0×10^{-4} M, respectively; Figure S10: (a–d) Binary mixtures of rutin and quercetin: (a) chemical structures; (b) SERS spectra; (c) composition ratios; (d) expanded spectral regions (550–650, 1380–1470, and 1500–1650 cm^{-1}). Binary mixtures were prepared at molar ratios of 4:1, 3:2, 2:3, and 1:4, corresponding to individual concentrations of 8.0×10^{-4} and 2.0×10^{-4} M, 6.0×10^{-4} and 4.0×10^{-4} M, 4.0×10^{-4} and 6.0×10^{-4} M, and 2.0×10^{-4} and 8.0×10^{-4} M, respectively; Figure S11: Extracted ion chromatogram and mass chromatograph of Northern Isatis Root (Heilongjiang); Figure S12: Extracted ion chromatogram and mass chromatograph of Southern Isatis Root (Sichuan); Figure S13. HPLC Chromatograms of Crude Extracts from Northern (Heilongjiang) and Southern (Sichuan) Isatis Root; Figure S14. Loading plots of principal components from LDA analysis of Isatis Root samples. (a,b) Loading plots for northern Isatis Root samples. (c,d) Loading plots for southern Isatis Root samples. (e,f) Loading plots for Isatis Root samples with different geographical origins. Table S1: Regions and assignments of characteristic bands of flavones [1–3]; Table S2: Regions and assignments of characteristic bands of isoflavones [4,5]; Table S3. Regions and assignments of characteristic bands of flavanones [6,7]; Table S4. Regions and assignments of characteristic bands of flavonols [8,9]; Table S5. Regions and assignments of characteristic bands of flavanols [10,11]; Table S6. Regions and assignments of characteristic bands of chalcones [12,13]. References [40–48] are cited in supplementary materials.

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