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Optimization and Efficacy Evaluation of NADES-Based Green Extraction for Total Flavonols from Eleutherine plicata Herb

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ABSTRACT

Flavonols in Eleutherine plicata have significant pharmacological value, but traditional extraction is inefficient and toxic. A green NADES system (3 sugars as HBDs, 2 acids as HBAs) was screened, characterized, and optimized by single-factor/orthogonal tests. Xylose-citric acid NADES performed best (16.81%, typical NADES properties). Optimized conditions: xylose/citric acid 1:4, 15% water, 1:2 solid-liquid ratio, 50℃/50 min. Validation yielded 24.42% (RSD=1.96%), far exceeding traditional methods (≈1.97%), and stable. This system solves the low-efficiency issue and provides a reference for natural flavonoid extraction.

KEYWORDS

Natural deep eutectic solvent (NADES); Eleutherine plicata Herb; Total flavonols; Extraction optimization

1. INTRODUCTION

Otanical medicines have gained increasing attention for chronic disease treatment and public health emergencies, with growing market share [1, 2]. Eleutherine plicata (Iridaceae, Allium genus) is widely used in traditional medicine for parasitic diseases, digestive disorders, etc., and modern pharmacology confirms its vasodilation, antibacterial, antioxidant, antitumor, and anti-inflammatory effects [3-5]. Previous studies focused on naphthols and quinones, while flavonol research is limited due to low traditional extraction rate (≈1.97%). Flavonols (kaempferol, isorhamnetin, quercetin, rutin) have anticancer, antioxidant, and immune regulatory activities consistent with E. plicata's pharmacological effects. Traditional extraction methods (ultrasonic, alcohol, water, ionic liquids) have drawbacks like degradation, low efficiency, or toxicity [6].

Deep eutectic solvents (DES) were reported in 2003, and natural deep eutectic solvents (NADES) in 2011. NADES is ideal for flavonol extraction due to good solubility for cross-conjugated compounds [7-10]. This study established a NADES-based green extraction system for E. plicata total flavonols.

2. MATERIALS AND METHODS

2.1. Samples and Reagents

Eleutherine plicata was collected from Puer, Yunnan. Cleaned and depurated, samples were dried at 50°C for 7 days to constant weight, then crushed and sealed. Reagents: pure-grade flavonol standards (rutin, quercetin, isorhamnetin, kaempferol), chromatographic-grade anhydrous methanol, analytical-grade glucose (≥99%), xylose/sucrose/citric acid/lactic acid (≥98%).

2.2. Flavonol Detection Method

Methanol-prepared flavonol standards were detected by HPLC. Key HPLC conditions: C18 reverse-phase column; mobile phase methanol (A)-1% phosphoric acid (B); flow rate 0.5 mL/min; column temperature 35°C; detection wavelength 360 nm; injection volume 5 μL; elution gradient: 0.01-3 min (45% B) → 3-5 min (45%-48% B) → 5-10 min (48%-50% B) → 10-20 min (50% B).

2.3. NADES Combination Screening

Six NADES combinations (glucose/citric acid, sucrose/lactic acid, xylose/lactic acid, xylose/citric acid, sucrose/citric acid, glucose/lactic acid) were prepared at specified molar ratios (heated to homogeneous transparency). Stability was tested via 7 h standing at room temperature.

2.4. Flavonol Extraction Rate Comparison

Extraction: solid-liquid ratio 1:5, 60°C for 1 h; extract diluted 50 times for detection. Extraction rate (%) = (C×V×N/M)×100% (C: extract concentration, μg/mL; V: constant volume, mL; N: dilution factor; M: sample mass, g).

2.5. NADES Property Characterization

Melting point, electrical conductivity, and refractive index of the optimal NADES were determined to confirm NADES properties [11].

2.6. Single-Factor Experiments

Effects of molar ratio (HBD: HBA), water content, solid-liquid ratio, extraction time, and temperature on total flavonol yield were examined.

2.7. Orthogonal Optimization Experiment

Based on single-factor results, a 5-factor-3-level orthogonal experiment (Table 3) optimized the system (variables: molar ratio, water content, solid-liquid ratio, extraction time, temperature).

3. RESULTS AND DISCUSSION

3.1. Validation of the Validity of the Detection Method for Flavonols

High-performance liquid chromatography (HPLC) successfully identified four target flavonols, with peaks 1–4 corresponding to rutin, quercetin, isorhamnetin, and shikonol respectively. All component peaks were fully separated, showing smooth chromatographic curves and stable baselines without significant tailing, which fully confirms the validity of the established detection method for flavonol analysis.

3.2. Preliminary Screening Results of HBDs and HBAs in NADES Combinations

Six NADES combinations (glucose/citric acid, sucrose/lactic acid, xylose/lactic acid, xylose/citric acid, sucrose/citric acid, glucose/lactic acid) were prepared at specified molar ratios, all forming clear and homogeneous liquids. After standing at room temperature for 7 hours, none of the combinations showed crystallization, turbidity or flocculation, maintaining good stability [12]. This indicates that the selected hydrogen bond donors (HBDs: glucose, sucrose, xylose) and hydrogen bond acceptors (HBAs: citric acid, lactic acid) meet the preparation requirements of NADES.

3.3. Comparison of Total Flavonol Extraction Rates from Eleutherine plicata Herb Using Different NADES Combinations

Extraction experiments showed that the six NADES combinations had significant differences in total flavonol extraction rates: NADES-1 (6.43%), NADES-2 (6.46%), NADES-3 (11.78%), NADES-4 (16.81%), NADES-5 (4.12%), and NADES-6 (14.18%). Among them, xylose-citric acid NADES (NADES-4) achieved the highest total extraction rate (16.81%), significantly exceeding other combinations. Though NADES-1 had the highest rutin extraction rate (4.25%), its total flavonol yield (6.43%) was less than 50% of NADES-4. Thus, NADES-4 was selected as the optimal solvent.

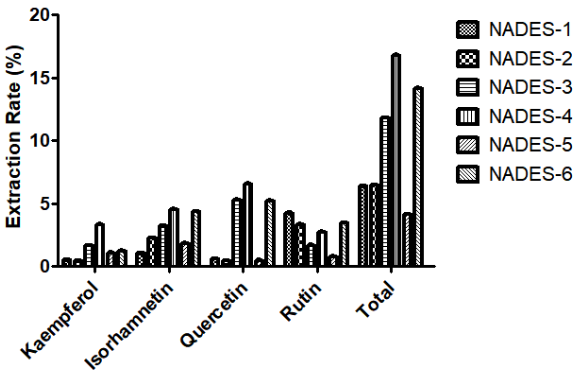

Figure 1. Comparison of Flavonol Extraction Rates Using NADES Groups

3.4. Determination of Physicochemical Properties of NADES-4

NADES-4's key properties confirmed its NADES identity (Figure 2): melting range 88–89°C (lower than xylose 114°C and citric acid 153–159°C); 50°C conductivity 88.9 µS/cm (close to conventional NADES range 0.1–10 mS/cm); refractive index >1.33 at all temperatures. Conductivity increased and refractive index decreased with temperature, consistent with NADES characteristics.

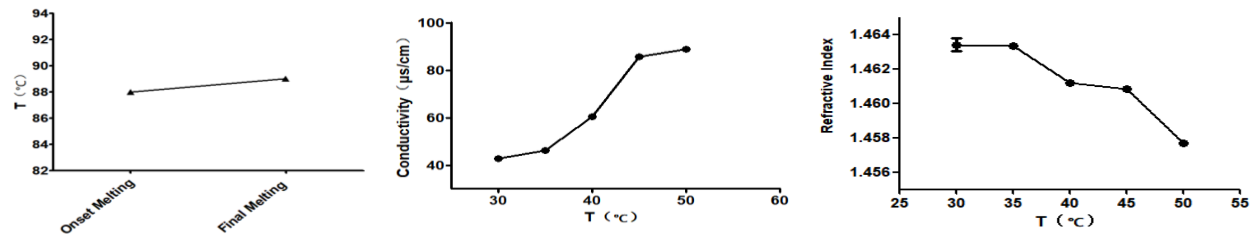

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Figure 2. Physical Property Verification of NADES-4 (Note: (a) Melting range tracking plot; (b) Conductivity measurement plot; (c) Refractive index measurement plot)

3.5. Optimization of the Green Extraction System for Total Flavonoids in Red Garlic NADES

Single-factor experiments showed molar ratio (HBD: HBA), water content, solid-liquid ratio, extraction time, and temperature all affected yield: optimal trends were molar ratio 1:5, water content 15%, solid-liquid ratio 1:3, time 50 min, temperature 45°C (Figures 3–7). A 5-factor-3-level orthogonal experiment identified the influence order: solid-liquid ratio > temperature > time > water content > molar ratio. Optimal conditions: xylose/citric acid 1:4, 15% water, solid-liquid ratio 1:2, 50°C for 50 min (Table 4). ANOVA confirmed solid-liquid ratio ($F=21.74$, $P<0.01$) and temperature

(F=16.87, P<0.01) were extremely significant, time (F=3.73, P<0.05) significant, and water content/molar ratio non-significant (Table 5).

Figures 3–7 Effects of Molar Ratio, Water Content, Solid-Liquid Ratio, Extraction Time, and Temperature on Total Flavonol Extraction Rate

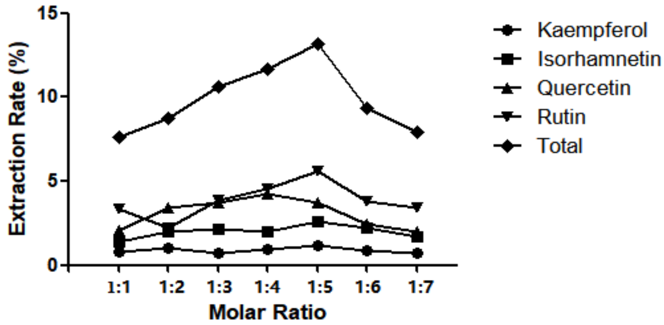

Figure 3. Effect of Molar Ratio of HBD to HBA on Total Flavonol Extraction Rates

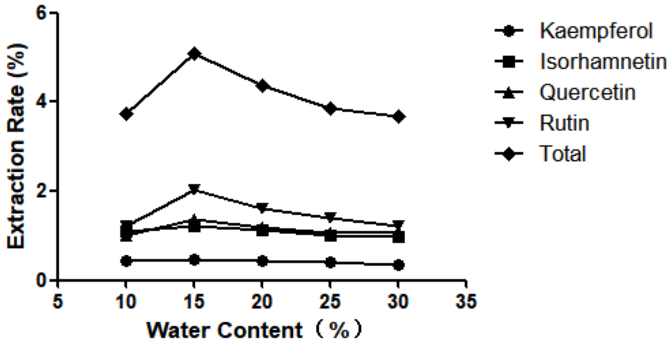

Figure 4. Effect of Water Content on Total Flavonol Extraction Rates

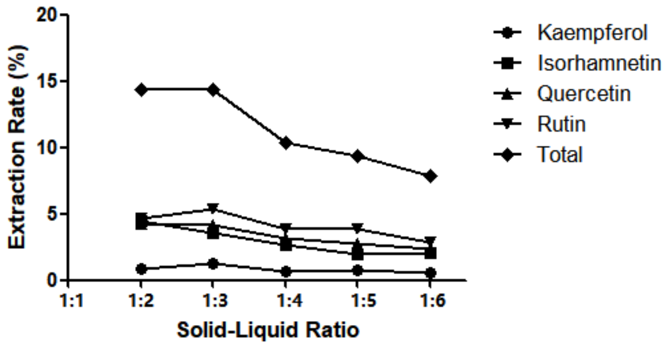

Figure 5. Effect of Solid-Liquid Ratio on Total Flavonol Extraction Rates

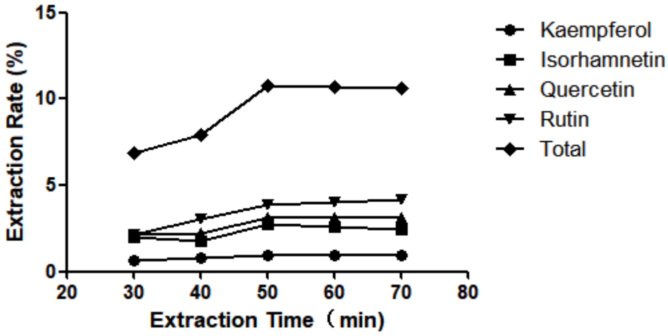

Figure 6. Effect of Extraction Time on Total Flavonol Extraction Rates

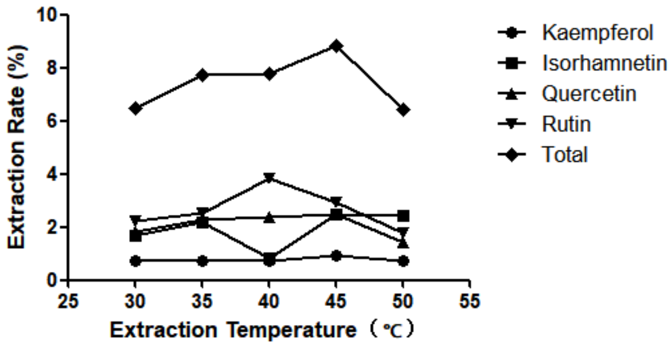

Figure 7. Effect of Extraction Temperature on Total Flavonol Extraction Rates

Single-factor experiments showed molar ratio (HBD: HBA), water content, solid-liquid ratio, extraction time, and temperature all affected yield: the extraction rate peaked at molar ratio 1:5, water content 15%, solid-liquid ratio 1:3, extraction time 50 min, and temperature 45°C. Based on this, a 5-factor-3-level orthogonal experiment was conducted (factors and levels: molar ratio 1:4/1:5/1:6, water content 10%/15%/20%, solid-liquid ratio 1:2/1:3/1:4, extraction time 40/50/60 min, temperature 40/45/50°C). Mean response analysis revealed the influence order: solid-liquid ratio > temperature > time > water content > molar ratio. The optimal conditions were confirmed as xylose/citric acid molar ratio 1:4, 15% water content, solid-liquid ratio 1:2, 50°C for 50 min. ANOVA results showed solid-liquid ratio ($F=21.74$, $P<0.01$) and temperature ($F=16.87$, $P<0.01$) had extremely significant effects, extraction time ($F=3.73$, $P<0.05$) had significant effect, while water content and molar ratio had no significant effects, verifying the orthogonal design's reliability.

3.6. Optimization System Verification and NADES Property Characterization

Validation experiments showed the optimized system achieved an average total flavonol extraction rate of 24.42% (RSD=1.96%), confirming high efficiency and precision. The optimized NADES retained typical characteristics: melting point lower than single components, 50°C conductivity 0.1116 mS/cm (within 0.1–10 mS/cm), refractive index >1.46 (temperature-dependent decrease, Figure 8). This verified the system’s scientific rationality.

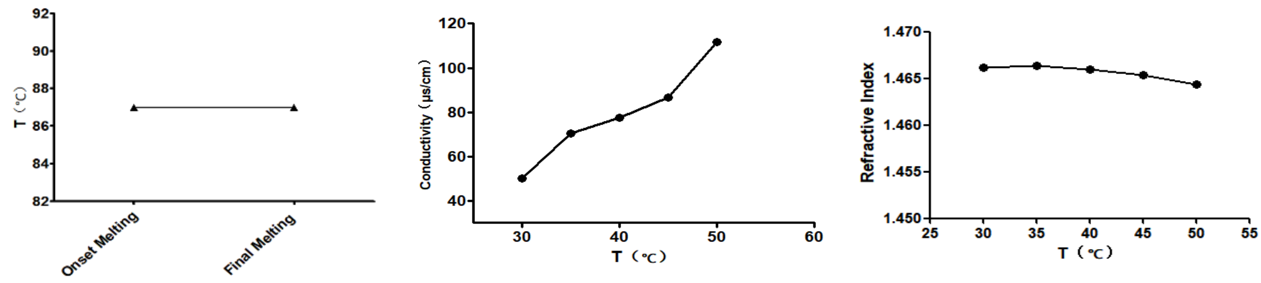

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Figure 8. Verification of physical properties of optimized red garlic total flavonoids (NADES)

Note: (a) Melting range tracking chart, (b) Conductivity measurement chart, (c) Refractive index measurement chart

3.7. Conclusion

This study aimed to establish a green natural deep eutectic solvent (NADES) extraction system for total flavonols from Eleutherine plicata Herb, achieving the research objectives by screening NADES combinations, optimizing extraction processes, and verifying solvent properties. Six NADES combinations were designed (glucose/citric acid 1:5, sucrose/lactic acid 1:3, xylose/lactic acid 1:3, xylose/citric acid 1:5, sucrose/citric acid 1:5, glucose/lactic acid 1:3). All prepared solvents were transparent liquids without abnormalities such as crystallization or flocculation, and remained stable after standing at room temperature for 7 hours, making them suitable for the extraction of natural plant components.

The total extraction rate of four major flavonols (kaempferol, isorhamnetin, quercetin, and rutin) was used as the evaluation index for total flavonols. Results showed that NADES-4 (xylose/citric acid 1:5) exhibited the highest total flavonol extraction rate, with its rutin extraction rate (2.73%) also significantly higher than that of traditional methods (1.97%) [13, 14]. Although NADES-1 (glucose/citric acid 1:5) achieved a high rutin extraction rate of 4.25%, its total flavonol extraction rate was less than 50% of that of NADES-4, making it only suitable for the specific extraction of rutin. The physical properties of NADES-4 conformed to the characteristics of NADES, and after optimization, the total flavonol extraction rate reached 24.42%, further confirming the superiority of this green extraction system.

This study only screened combinations of 3 sugar-based HBDs and 2 acid-based HBAs. The selected solvents possess the advantages of low cost, easy accessibility, high extraction efficiency, and environmental friendliness, demonstrating broad application prospects in biomedicine, food processing, and natural product extraction.

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REFERENCES

[1] Nam Y H, Ahn S M, Seo G J, et al. Optimization of Nades-Based Green Extraction of Ellagitannins from Rambutan Peel with Enhanced Antioxidant Activity [J]. 2024.

[2] Karabulut G, Unlu A, Demirer S, et al. RuBisCO-based protein and film from Rumex patience L.: Effects of green extraction and NADES plasticization on structural and functional properties [J]. Food Chemistry, 2025, 493(Part4).

[3] Miloevi S, Markovinovi A B, Tesli N, et al. Use of natural deep eutectic solvent (NADES) as a green extraction of antioxidant polyphenols from strawberry tree fruit (Arbutus unedo L.): An optimization study [J]. Microchemical Journal: Devoted to the Application of Microtechniques in all Branches of Science, 2024:200.

[4] Ji D, Brooks S L. Enhancing bio-based polysaccharide/protein film properties with Natural Deep Eutectic Solvents (NADESs) and NADES-based bioactive extracts – A review [J]. Future Foods, 2025, 11.

[5] Mahrous R S, Fattah B A, Rahman Y A, et al. Green and Efficient Extraction of Soybean (Glycine max (L.) Merr.) Isoflavones Using Natural Deep Eutectic Solvents-Based Ultrasound-Assisted Extraction [J]. Food and Bioprocess Technology, 2025, 18(12):10419-10433.

[6] Wang D, Kang L, Hamdi M, et al. Advanced ultrasounds-assisted natural deep eutectic solvents green strategy for the sustainable extraction of biocompatible nano-hydroxyapatite from fish scales [J]. Journal of Molecular Liquids, 2025, 428(000).

[7] Oliva E, Mir-Cerdà, Aina, Sergi M, et al. Green extraction of phenolic compounds from strawberry waste based on natural deep eutectic solvents [J]. International Journal of Food Science & Technology, 2024, 59(6).

[8] Kaur D, Qadri O S. Green extraction of anthocyanins from Syzygium cumini fruit pulp using aqueous glycerol through ultrasound-assisted extraction [J]. Umm Al-Qura University Journal of Applied Sciences (Springer Nature), 2025, 11(1).

[9] Julie Q, William B, Solenn F, et al. Alternatives for the extraction of bioactives and biopolymers from Evernia prunastri for the formulation of antimicrobial bio-based films [J]. Green chemistry, 2024(19):26. DOI:10.1039/D4GC02741H.

[10] Rashid S N, Hizaddin H F, Hayyan, AdeebChan, Shee EnHasikin, KhairunnisaRazak, Sarah AbdulMokhtar, Mohd IstajibAzizan, Muhammad Mokhzaini.A kinetic study of ex-situ soil remediation by nickel extraction using natural deep eutectic solvent [J]. Environmental technology, 2024, 45(21/24):4820-4833.

[11] Carbonell-Rozas L, Canales R, Roberto Romero-González, et al. Structural characterization and physicochemical properties of different hydrophilic natural deep eutectic solvents [J]. Analytical and Bioanalytical Chemistry, 2025, 417(1):183-197.

[12] Heredia M B J B, emailprotected, * emailprotected,et al.Optimized Extraction of Glycosylated Flavonoids from Pepper (Capsicum annuum L.) Agricultural Biomass Residues through Ultrasonic Pulse-Assisted Natural Deep Eutectic Solvents [J]. ACS Sustainable Resource Management, 2024, 1(1):165-177..

[13] Nikodijevi M, Troter D, Konstantinovi S. Dyeing of polyester fabric using a natural deep eutectic solvent [J]. Advanced technologies, 2024, 13(2):45-52.

[14] Soukaina K, Safa Z, Soukaina H, et al. Choline chloride-based deep eutectic solvents (NADES): Potential use as green extraction media for polyphenols from Mentha pulegium, antioxidant activity, and antifungal activity [J]. Microchemical Journal, 2024, 199:110174.