Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS
Pharmacy

A Review on the Extraction of Total Flavonoids from Liubao Tea Residue and Its Antioxidant Activity

Sun Gongbing[1], Yuan Yuan[1], Qin Jingwen[2], Yuan Yuping[2]*

[1]School of Food and Pharmaceutical Engineering, Wuzhou university, Guangxi Wuzhou 543002, China
[2]School of Electronic Information & Artificial Intelligence, Wuzhou University, Guangxi Wuzhou 543002, China

DOI: https://doi.org/10.36347/sajp.2026.v15i11.001 | **Received:** 23.08.2026 | **Accepted:** 29.10.2026 | **Published:** 09.11.2026

***Corresponding author:** Yuan Yuping
School of Electronic Information & Artificial Intelligence, Wuzhou University, Guangxi Wuzhou 543002, China

Abstract | **Review Article**

Liupao tea is a post-fermented dark tea produced only in Wuzhou, Guangxi. Substantial quantities of tea residue are generated during its processing and consumption, and this residue is rich in flavonoids, making it a potential source of natural antioxidants. This review summarises recent progress in the extraction and antioxidant evaluation of total flavonoids from Liupao tea residue. Organic solvent extraction, deep eutectic solvent (DES) extraction, ultrasound- and microwave-assisted extraction, and enzyme-assisted extraction are compared in terms of their operating principles and practical suitability. The viscosity and polarity of DES emerge as the decisive factors governing extraction efficiency, and the combination of ternary DES with ultrasound is shown to raise flavonoid yield markedly while shortening processing time. The merits and limitations of DPPH·, $ABTS^{+}$· and hydroxyl radical scavenging assays, together with those of cellular models, are then assessed, and the structural features that determine activity the number and position of phenolic hydroxyl groups, the o-dihydroxy group on the B ring, the C2 C3 double bond of the C ring, and glycosylation are discussed. Finally, taking account of the oxidative polymerisation of catechins during pile fermentation, standardisation of raw materials, scale-up of the process and comprehensive characterisation of components are proposed as the main directions for future research.

Keywords: Liupao tea residue; total flavonoids; extraction; antioxidant activity.

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Liupao tea is a post-fermented dark tea unique to Wuzhou, Guangxi. Its traditional manufacturing technique was inscribed on the UNESCO Representative List of the Intangible Cultural Heritage of Humanity in 2023. As the industry has expanded, the tea residue generated during processing and consumption has come to represent some 15%–25% of the dry weight of the raw material. Most of this residue is currently landfilled or incinerated, and its flavonoid content remains unexploited.

Flavonoids are among the most important secondary metabolites in tea. They exert antioxidant effects by donating hydrogen atoms or electrons, chelating metal ions and inhibiting lipid peroxidation. Pile fermentation of Liupao tea causes extensive oxidative polymerisation of catechins [1], giving a product profile that differs markedly from that of green tea; transmission electron microscopy and metabolomic studies have revealed how cell wall structure and the metabolic profile evolve during fermentation [2-4]. Recovering such compounds from tea residue depends on two things: an efficient green extraction technique, and reliable evaluation of the resulting activity. Research to date has focused largely on green tea, black tea and fresh leaves, and systematic reviews dealing specifically with post-fermented tea residue remain scarce. This review therefore examines recent progress in extraction technology and antioxidant evaluation over the past five years, and relates it to the compositional changes brought about by pile fermentation, with the aim of supporting the valorisation of Liupao tea residue.

1 Characteristics of the Raw Material

Compared with fresh tea leaves, Liupao tea residue differs in three main respects. First, pile fermentation alters its composition: microbial enzymes drive the extensive oxidative polymerisation of esterified catechins into theaflavins, thearubigins and theabrownins [1], so that free catechins decline and polymeric phenolics rise. Second, the tissue structure is disrupted: pile fermentation damages cell walls and

membranes and changes how active components are bound to the matrix [2], which aids solvent penetration but also renders the components more susceptible to oxidation. Third, brewing leaches out the soluble fraction: the more water-soluble flavonoid glycosides pass preferentially into the infusion, leaving behind mainly bound flavonoids and hydrophobic aglycones, while the dense fibrous structure that remains after drying further impedes penetration by conventional solvents.

These features make flavonoid extraction from tea residue more demanding than from ordinary plant materials, since the solvent must dissolve components of widely differing polarity while also disrupting a compact matrix. They explain why conventional ethanol reflux performs poorly on this material, whereas green solvents and physically assisted techniques show a clearer advantage [5-6].

2 Extraction Technologies

2.1 Organic solvent extraction

Organic solvent extraction comprises reflux, Soxhlet and maceration. It relies on the principle that like dissolves like; ethanol is the most widely used solvent, typically at a volume fraction of 60%–80%, a temperature of 70–90 °C and a duration of 1–3 h. The method requires only simple equipment, is easy to scale up and extracts aglycones reasonably well. Its drawbacks are equally clear: the high temperature and long duration promote degradation of heat-sensitive components, solvent consumption is high, and efficiency for strongly polar flavonoid glycosides and bound flavonoids is limited. In the dense fibrous matrix of tea residue, solvent penetration alone cannot release the target compounds efficiently [2], so the method now serves mainly as a benchmark or in combination with other techniques.

2.2 Deep eutectic solvent extraction

A deep eutectic solvent (DES) forms when a hydrogen-bond acceptor and a hydrogen-bond donor assemble through hydrogen bonding in a defined molar ratio. Type III DESs, in which choline chloride acts as the acceptor and an organic acid or a polyol as the donor, are the most widely used because they are of low toxicity and readily biodegradable [7-8]. DESs have low melting points, negligible vapour pressure and are non-flammable, and are therefore regarded as green media capable of replacing volatile organic solvents.

Flavonoids are extracted by DESs through three mechanisms. Hydrogen bonding allows the donor to form multiple hydrogen bonds with the phenolic hydroxyls of the flavonoid, lowering the energy barrier for transfer from the solid to the liquid phase. Ionic interaction allows the quaternary ammonium cation, through cation–π interaction with the electron-rich π system of the B ring, to compensate for the limited ability of purely hydrogen-bonded systems to dissolve low-polarity aglycones. Polarity can be tuned by varying the identity and ratio of acceptor and donor and by adjusting the water content, so that the polarity and viscosity of the system can be controlled systematically. Solvent polarity has been shown to be decisive for extraction selectivity, and tailoring the DES composition to the target compound markedly improves efficiency [5,8].

Viscosity is the physical property that most strongly affects DES performance. Most choline chloride-based DESs have a room-temperature viscosity of tens to hundreds of millipascal-seconds, which markedly slows mass transfer. The usual strategies for reducing viscosity are heating, adding water and introducing low-viscosity components. The effect of water content is twofold: a moderate amount of water disrupts the internal hydrogen-bond network, lowers viscosity and increases polarity so as to favour dissolution of glycosides, whereas excess water drives the properties of the DES towards those of an ordinary aqueous solution and actually reduces its selectivity for moderately polar components [9]. Introducing a second hydrogen-bond donor to form a ternary DES yields a richer and more highly cross-linked hydrogen-bond network, with a wider range of polarity adjustment and a lower tendency to crystallise [7]. Reports of ternary DESs in flavonoid extraction are, however, still limited: the space of possible compositions is large, and the underlying synergistic mechanisms have yet to be resolved systematically.

DESs also allow product separation and solvent recovery through adjustment of water content or addition of an antisolvent, and the recyclability of the solvent directly determines the economics of the process; studies of recycling in flavonoid systems remain scarce, however [5]. In addition, organic-acid-based DESs pose a corrosion risk to equipment, so material compatibility and the cost of neutralisation must be assessed before industrial application.

2.3 Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) exploits the cavitation produced by ultrasound at 20–100 kHz: bubbles collapse violently, generating local extremes of temperature and pressure together with microjets that disrupt cell walls, increase the contact area and accelerate the diffusion of intracellular components [10-11]. Compared with reflux, UAE achieves a comparable or higher yield at a lower temperature and in a shorter time, with less degradation of heat-sensitive components. The parameters that govern efficiency include power, frequency, temperature, time, liquid-to-solid ratio and particle size. Too little power gives insufficient cavitation, whereas too much can oxidise already-dissolved components through the reactive oxygen species generated by cavitation; excessive duration fragments the cells into a colloidal suspension that is difficult to separate. Optimisation is therefore usually carried out by single-factor experiments combined with

response surface methodology. When UAE was applied to polyphenol extraction from spent tea, combining ultrasound with a conventional method outperformed either method alone [6], indicating that tea residue is better handled by combined approaches.

2.4 Synergy between ultrasound and DES

UAE and DES are complementary: the high viscosity of the DES restricts mass transfer, while ultrasonic microjets intensify the penetration and renewal of the DES within the pores of the matrix. In foxtail millet bran, apple pomace, olive leaves, Chinese bayberry leaves and vine tea, ultrasound-assisted DES extraction has given higher yields and shorter equilibration times than DES maceration alone or conventional solvent extraction [9,12-15]. These studies generally optimised the DES composition, water content, liquid-to-solid ratio, temperature, time and ultrasonic power together by response surface methodology, which indicates that the parameter space of the combined process is large and that superimposing single-factor optima is unlikely to locate the true optimum. Some studies have also found that the activity of the product is not compromised when the yield is raised [13,15], suggesting that efficient extraction under mild conditions helps to preserve the active components.

2.5 Microwave- and enzyme-assisted extraction

Microwave-assisted extraction relies on the selective internal heating of polar molecules, which vaporises intracellular water and generates internal pressure that ruptures the cell wall. It heats rapidly, is quick and uses little solvent, but the heating is uneven and can cause local overheating and degradation. Enzyme-assisted extraction uses cellulases, pectinases and related enzymes to hydrolyse cell wall constituents and release bound flavonoids; it is mild, selective and solvent-free, but for tea residue that has already been partly disrupted the marginal gain over untreated material may be smaller than for fresh raw material, the enzymes are costly, and temperature and pH must be controlled closely. Coupling enzymatic hydrolysis with ultrasound or DES is a feasible route to higher overall efficiency.

Table 1: Comparison of the main technologies for extracting total flavonoids from Liupao tea residue

Technique	Principle	Typical conditions	Advantages	Limitations
Organic solvent	like dissolves like; thermally driven diffusion	60%–80% ethanol, 70–90 °C, 1–3 h	simple equipment; easy scale-up	time-consuming; high solvent use; heat-sensitive components degrade
DES	hydrogen bonding and ionic interaction; tunable polarity	choline chloride-based DES, water 10%–40%, 40–70 °C	green; designable polarity; recyclable	high viscosity; slow mass transfer; limited recycling data
Ultrasound-assisted	cavitation disrupts cell walls	20–40 kHz, 200–500 W, 20–60 min	rapid; low temperature; disrupts dense matrix	over-processing oxidises components; difficult separation
Ultrasound–DES	cavitation intensifies DES penetration	joint optimisation of ultrasonic and DES parameters	high yield; short processing time	large parameter space; uneven acoustic field on scale-up
Microwave-assisted	selective internal heating ruptures cells	300–800 W, several minutes	very fast; little solvent	uneven heating; high equipment demand
Enzyme-assisted	enzymatic hydrolysis releases bound forms	cellulase etc., 40–55 °C, pH 4–6	mild; selective; solvent-free	enzyme cost; strict control of temperature and pH

3 Methods for Evaluating Antioxidant Activity

In terms of reaction mechanism, in vitro chemical assays fall into two groups: those based on hydrogen atom transfer (HAT) and those based on single electron transfer (SET). The former measures the ability to donate a hydrogen atom to a radical, the latter the reducing capacity; both operate in most systems and differ only in their relative contribution [16]. The DPPH· assay uses the 1,1-diphenyl-2-picrylhydrazyl radical as a probe and follows the decrease in absorbance at 517 nm as a measure of scavenging capacity. It is simple, reproducible and the most widely used preliminary screen, but DPPH· is a bulky, hydrophobic radical that does not come into full contact with strongly polar flavonoid glycosides, and the reaction reaches equilibrium slowly and is sensitive to solvent and pH [17]. The $ABTS^{+\cdot}$ assay uses the ABTS radical cation as a probe; it is soluble in both aqueous and organic media, proceeds mainly by electron transfer, is fast and is little affected by steric hindrance, so its scope is broader. Hydroxyl radical (·OH) scavenging assays generally generate ·OH in situ by the Fenton reaction; because ·OH has a high oxidation potential (about 2.80 V), an extremely short lifetime and no selectivity, scavenging depends largely on whether the antioxidant can intercept ·OH before it diffuses to other substrates. The ORAC assay, which follows fluorescence decay, is a typical HAT-based method and is applicable to both hydrophilic and lipophilic samples [16].

Because these methods differ in mechanism, medium, pH and time, their results are often not interconvertible. For an extract as complex as that from tea residue, several assays should therefore be run in parallel and interpreted together with a dose–response measure such as the IC_{50}. In vitro chemical assays cannot reflect absorption, distribution or metabolism in vivo; cellular antioxidant activity (CAA) assays and animal studies fill this gap. The bioavailability of flavonoids is generally low, however, and they are metabolised in vivo

to conjugates such as glucuronides, so inferring in vivo efficacy from in vitro results alone is risky [18].

4 Structure–Activity Relationships

The antioxidant activity of a flavonoid is determined by its molecular structure, above all by the number and position of its phenolic hydroxyl groups and by the surrounding conjugated system. Phenolic hydroxyls supply the active hydrogen, and activity generally rises with their number, but position matters more. The o-dihydroxy (catechol) group on the B ring forms a stable phenoxyl radical and stabilises the intermediate through an intramolecular hydrogen bond, making it the single most important structural unit; the 3-OH of the C ring extends the conjugated system and contributes positively. Quantitative structure–activity studies based on molecular simulation and machine learning show that the number and position of hydroxyl groups and the overall degree of conjugation together determine scavenging capacity [19], and in complex matrices the relationship is further modulated by co-existing components.

The conjugated system formed by the C2 C3 double bond of the C ring and the 4-carbonyl group favours delocalisation of the phenoxyl radical intermediate and lowers the activation energy for dehydrogenation. The effect of glycosylation is more complex and generally negative: the sugar increases polarity and water solubility but introduces steric hindrance and alters the intramolecular hydrogen-bond network, weakening the hydrogen-donating capacity of the B-ring hydroxyls, and comparative studies show that aglycones are usually more active than the corresponding O- and C-glycosides [20]. Esterified catechins carry galloyl groups that provide additional phenolic hydroxyls and give strong in vitro scavenging capacity, so their oxidative polymerisation during processing represents a substantial loss of active sites.

Existing structure–activity studies mostly use single compounds measured in a single system, and their ability to extrapolate to complex extracts is limited: co-existing components may act synergistically or antagonistically, pigments, polysaccharides and proteins interfere with the measurement, and most models use only two-dimensional descriptors that capture three-dimensional features poorly [19]. Such models should therefore be applied to extracts only within a clearly stated domain, and a more workable approach is to discuss structural effects together with quantitative data on the composition of the individual compounds.

5 Effect of Pile Fermentation on Flavonoid Activity

Pile fermentation is the key step that determines the flavonoid composition and activity of tea residue. Studies have elucidated the route by which epicatechin oxidation dimers are formed by crude tea enzymes and its link to thearubigin formation [1], and have shown that the succession of the microbial community is closely synchronised with changes in catechin content [4]. The transformation affects activity in two opposing ways. On the one hand, oxidation of free esterified catechins markedly reduces the number of o-dihydroxy sites on the B ring, and these are precisely the most important structural units. On the other, theaflavins, thearubigins and other polymeric products retain some phenolic hydroxyls and therefore some radical scavenging capacity. The net outcome depends on the extent of fermentation: moderate pile fermentation reconciles flavour with activity, whereas over-fermentation leads to a clear decline in activity.

Tea residue has in addition undergone a second selection through leaching during brewing, so that the residual components are biased towards low polarity and the bound form. It follows that lightly fermented residue, such as screening fragments and trimmings, may retain more highly active catechins and deserves to be treated separately when raw materials are selected.

6 Remaining Problems and Future Prospects

First, systematic work on tea residue as a specific matrix is lacking. Green extraction techniques have mostly been validated on foxtail millet bran, apple pomace and olive leaves [9,12,14-15], and few studies have addressed Liupao tea residue directly, even though cell wall structure and component polarity differ greatly between matrices and processing parameters cannot simply be transferred. Future work should compare the yield, product activity and cost of each technique on tea residue, using a uniform batch of raw material and a standardised pretreatment.

Second, the active components have not been characterised in sufficient depth. Most studies describe results in terms of total flavonoid content and total antioxidant capacity, with little quantitative account of individual composition, polymeric distribution or their relationship to activity. High-performance liquid chromatography–mass spectrometry and related techniques should be used to establish a compositional profile of tea residue flavonoids, so that structure–activity discussion rests on measured composition.

Third, engineering data for green processes are scarce. Data at pilot scale are lacking on the recyclability of DESs, on compositional drift and impurity accumulation over repeated cycles, on the uniformity of the acoustic field when ultrasound is scaled up, and on corrosion of equipment by acid-containing DESs [5]. Fourth, the evaluation system needs to be improved. In vitro chemical assays currently dominate, results from different assays are difficult to compare, and they are disconnected from in vivo effects [18]. Reports of scavenging rates or IC_{50} values should specify the assay conditions and the reference system, and cellular models should be introduced where appropriate.

Acknowledgments

This work was financially supported by theWuzhou Municipal Science and Technology Programme “Extraction of total flavonoids from Liupao tea residue and their antioxidant activity” （Project No. 202402026); Wuzhou University Talent Introduction Research Start-up Fund “Twin support vector machine and its application in hydrological forecasting” （Project No. WZUQDJJ17143).

REFERENCES

1. WEN M C, LI L, HU W, *et al.,* Epicatechin oxidation dimers produced by crude tea enzymes: isolation, structural elucidation, and potential link to thearubigin formation[J]. Journal of Agricultural and Food Chemistry, 2026, 74(30): 23726-23740.
2. PANG B W, HUANG L, TENG J W, *et al.,*Effect of pile fermentation on the cells of Chinese Liupao tea: the first record of cell wall of Liupao tea on transmission electron microscope[J]. Food Chemistry, 2021, 361: 130034.
3. WANG H F, TENG J W, HUANG L, *et al.,*Determination of the variations in the metabolic profile and sensory quality of Liupao tea during fermentation through UHPLC-HR-MS metabolomics[J]. Food Chemistry, 2023, 404: 134773.
4. LONG J Y, CHEN C, WANG Y Q, *et al.,*Exploring the microbial community, physicochemical properties, metabolic characteristics, and pathways during tank fermentation of Liupao tea[J]. LWT, 2024, 204: 116449.
5. BEZERRA F S, KOBLITZ M G B. Extraction of phenolic compounds from agro-industrial by-products using natural deep eutectic solvents: a review of green and advanced techniques[J]. Separations, 2025, 12(6): 150.
6. OZSEFIL I C, ZIYLAN-YAVAS A. Green approach for polyphenol extraction from waste tea biomass: single and hybrid application of conventional and ultrasound-assisted extraction[J]. Environmental Research, 2023, 235: 116703.
7. RAN H L, LI H, PENG D L, *et al.,*Research progress on extraction of flavonoids with deep eutectic solvents from natural products[J]. Journal of Molecular Liquids, 2025, 426: 127356.
8. ALI REDHA A. Review on extraction of phenolic compounds from natural sources using green deep eutectic solvents[J]. Journal of Agricultural and Food Chemistry, 2021, 69(3): 878-912.
9. PONTES P V A, SHIWAKU I A, MÁXIMO G J, *et al.,*Choline chloride-based deep eutectic solvents as potential solvent for extraction of phenolic compounds from olive leaves: extraction optimization and solvent characterization[J]. Food Chemistry, 2021, 352: 129346.
10. YUSOFF I M, MAT TAHER Z, RAHMAT Z, *et al.,*A review of ultrasound-assisted extraction for plant bioactive compounds: phenolics, flavonoids, thymols, saponins and proteins[J]. Food Research International, 2022, 157: 111268.
11. KUMAR K, SRIVASTAV S, SHARANAGAT V S. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: a review[J]. Ultrasonics Sonochemistry, 2021, 70: 105325.
12. LV J M, ZHOU W T, SUN C D, *et al.,*Ultrasound-assisted deep eutectic solvent extraction of flavonoids from Chinese bayberry leaves: optimization, molecular mechanism, and antioxidant evaluation[J]. Food Chemistry, 2026, 512: 148996.
13. CAI X, XIONG Y G, SUN Q Y, *et al.,*Ultrasound-assisted deep eutectic solvents extraction of flavonoids from vine tea: solvent selection, optimization process, extraction mechanism and antioxidant activity in vitro[J]. Journal of Molecular Liquids, 2026, 441: 128941.
14. ZHENG B L, YUAN Y, XIANG J L, *et al.,*Green extraction of phenolic compounds from foxtail millet bran by ultrasonic-assisted deep eutectic solvent extraction: optimization, comparison and bioactivities[J]. LWT, 2022, 154: 112740.
15. RASHID R, MOHD WANI S, MANZOOR S, *et al.,*Green extraction of bioactive compounds from apple pomace by ultrasound assisted natural deep eutectic solvent extraction: optimisation, comparison and bioactivity[J]. Food Chemistry, 2023, 398: 133871.
16. TAKATSUKA M, GOTO S, KOBAYASHI K, *et al.,*Leading individual features of antioxidant systematically classified by the ORAC assay and its single electron transfer and hydrogen atom transfer reactivities[J]. BBA Advances, 2021, 1: 100030.
17. GULCIN İ, ALWASEL S H. DPPH radical scavenging assay[J]. Processes, 2023, 11(8): 2248. DOI: 10.3390/pr11082248.
18. SHAHIDI F, SAMARASINGHE A. How to assess antioxidant activity? Advances, limitations, and applications of in vitro, in vivo, and ex vivo approaches[J]. Food Production, Processing and Nutrition, 2025, 7(1): 39.
19. LU L, LUAN Y J, WANG H Q, *et al.,*Flavonoid as a potent antioxidant: quantitative structure-activity relationship analysis, mechanism study, and molecular design by synergizing molecular simulation and machine learning[J]. The Journal of Physical Chemistry A, 2024, 128(30): 6216-6228.
20. XIE L Q, DENG Z Y, ZHANG J, *et al.,*Comparison of flavonoid O-glycoside, C-glycoside and their aglycones on antioxidant capacity and metabolism during in vitro digestion and in vivo[J]. Foods, 2022, 11(6): 882.