JTE HCM-UTE
ISSN: 2615-9740

JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Engineering
Website: https://jte.edu.vn
Email: jte@hcmute.edu.vn

Phytochemical Profiling and Antioxidant Activity of *Ficus auriculata* Leaf Extracts From Three Vietnamese Regions: Solvent Polarity, Geographical Variation

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ARTICLE INFO

Received: 26/04/2026
Revised: 23/05/2026
Accepted: 11/06/2026
Online First: 03/09/2026
Published:

KEYWORDS

Ficus auriculata;
Phytochemical profiling;
HPLC–MS–ESI;
Antioxidant activity;
DPPH;
FRAP.

ABSTRACT

This study investigates the phytochemical composition and biological activities of *Ficus auriculata* leaf extracts obtained from three geographical regions of Vietnam (Hue, Dak Lak, and Dong Nai) using solvents of varying polarity (heptane, ethanol, and distilled water). Qualitative phytochemical screening, high-performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC–MS–ESI), DPPH radical scavenging, and ferric reducing antioxidant power (FRAP) assays were employed to characterize the extracts. Polar extracts (ethanol and water) demonstrated superior phytochemical diversity, particularly in polyphenolic compounds such as flavonoids, tannins, and proanthocyanidins, correlating with markedly higher antioxidant activity. The Hue water extract (H-W) exhibited the strongest antioxidant activity (DPPH IC_{50} = 11.28 ± 0.22 µg/mL; FRAP EC_{50} = 4.89 ± 0.08 µg/mL). Geographical origin further modulated phytochemical accumulation, with the Dong Nai water extract (DN-W) showing notably elevated polyuronic compound levels. These findings provide a scientific basis for the development of *F. auriculata*-derived natural antioxidant agents for environmental and biomedical applications.

DOI: https://doi.org/10.54644/jte.2026.2389

1. Introduction

Naturally derived compounds, particularly plant secondary metabolites such as polyphenols, flavonoids, and terpenoids, have attracted significant attention owing to their diverse biological activities, including antioxidant, antibacterial, antifungal, anti-inflammatory, anticancer, and antiviral activities. These compounds play a crucial role in scavenging free radicals, thereby mitigating oxidative stress, which is a key factor in various chronic diseases and biological deterioration.

Ficus auriculata Loureiro (Moraceae), commonly known as the Roxburgh fig, is a tropical plant widely distributed in Southeast Asia, including Vietnam. Previous studies indicate that species of the Ficus genus are rich in bioactive phytochemicals with potential antioxidant activity [1]. However, comprehensive investigations examining the interplay between extraction solvent polarity, geographical origin, phytochemical composition, and biological activities of *F. auriculata* remain limited.

Variations in environmental conditions, including climate and soil characteristics, can significantly influence phytochemical profiles of plant materials. The choice of extraction solvent—ranging from non-polar (heptane) to polar (ethanol and distilled water)—is equally critical in determining the types and yields of extracted bioactive compounds [2].

In this study, *F. auriculata* leaf samples were collected from three regions in Vietnam: Hue (H), Dak Lak (DLK), and Dong Nai (DN). Extracts were prepared using heptane (He), ethanol (Et), and distilled water (W). Chemical composition was characterized by HPLC–MS–ESI; antioxidant activities were evaluated by DPPH and FRAP assays. To the best of our knowledge, this is among the first systematic comparisons of geographical and solvent effects on *F. auriculata* biological activity in Vietnam. The

rest of this paper is organized as follows: Section 2 describes materials and methods; Section 3 presents results and discussion; Section 4 provides conclusions.

2. Materials and Methods

2.1. Chemicals and Plant Materials

2.1.1. Chemicals

All chemicals and reagents used in this study were of analytical grade and used without further purification. Methanol, ethanol, heptane, formic acid, ferric chloride ($FeCl_3$), potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid (TCA), and other solvents were purchased from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.1.2. Plant Materials

F. auriculata leaves were collected in November 2025 from three provinces: Hue (Central Vietnam), Dak Lak (Central Highlands), and Dong Nai (Southeast Vietnam). The plant material was authenticated before analysis. The dried leaf powder (25 g) was extracted with 150 mL of three different solvents (heptane, ethanol, and distilled water) using a round-bottom flask heating system. The resulting extracts were concentrated using a vacuum rotary evaporator at 40–45 °C to obtain crude extracts. For chemical analysis, 10 mg of each crude extract was dissolved in 1 mL of HPLC-grade methanol, ultrasonicated until complete dissolution, and filtered through a 0.22 µm PTFE membrane. A total of 9 samples, derived from three geographical regions and three extraction solvents, were stored in 2 mL vials at 4 °C for further investigation.

2.2. Qualitative Phytochemical Screening

Qualitative phytochemical screening was performed on crude extracts to detect major classes of secondary metabolites, including phenolic compounds, flavonoids, alkaloids, saponins, tannins, triterpenoids, and polyuronic compounds. Each test employed standard colorimetric or precipitate-based reactions, and results were recorded as the presence or absence (with intensity grade) of each compound class [3].

2.3. HPLC–MS–ESI Analysis

Chemical constituents were analyzed using an HPLC system coupled to mass spectrometry with an electrospray ionization (ESI) source [4]. Chromatographic separation was performed on a C18 reversed-phase column (4.6 × 250 mm) using a mobile phase of methanol and 0.1% (v/v) formic acid in distilled water at a flow rate of 1.0 mL/min. Mass spectra were compared with literature data and spectral databases (ReSpect) for compound identification [5].

2.4. DPPH Radical Scavenging Assay

The free radical scavenging activity of the extracts was assessed using a modified DPPH assay based on a previously reported microplate method [6]. A 150 µM DPPH solution was freshly prepared in methanol and stored in the dark until use.

Extract stock solutions were prepared at a concentration of 12 mg/mL in 50% methanol and then serially diluted to create a range of concentrations. An aliquot of 100 µL from each sample was combined with 100 µL of the DPPH solution in a 96-well plate. The control consisted of the DPPH solution with only the solvent, while the blank contained only the solvent.

The mixtures were incubated in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm using a microplate reader [7]. The radical scavenging activity was calculated as the percentage of inhibition relative to the control. IC_{50} values (in µg/mL) were determined by nonlinear regression analysis of the dose–response curves, with ascorbic acid serving as a positive control [6], [8].

2.5. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP of the extracts was evaluated using a modified method based on the protocol by Benzie and Strain [9]. This assay measures the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} under acidic conditions.

In brief, the reaction mixture consisted of the sample solution, phosphate buffer (pH 6.6), and a 1% (w/v) solution of potassium ferricyanide. After incubating at 50 °C for 20 minutes, 10% (w/v) trichloroacetic acid was added to stop the reaction. The mixture was then diluted with distilled water and combined with a 0.1% (w/v) $FeCl_3$ solution [10].

The absorbance was measured at 593 nm using a UV–Vis spectrophotometer. The reducing power of the extracts was expressed as EC_{50}, which is defined as the concentration needed to achieve an absorbance of 0.5 (or 50% of the maximum absorbance). All measurements were conducted in triplicate [9].

2.6. Statistical analysis

All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation (SD). Data processing and graphical representation were performed using OriginPro (OriginLab, USA) and Microsoft Excel.

3. Results and Discussion

3.1. Qualitative Phytochemical Screening

The qualitative phytochemical screening results are presented separately by region in Tables 1 (Dong Nai), 2 (Dak Lak), and 3 (Hue). Solvent polarity was the dominant factor determining extraction efficiency; geographical origin introduced secondary, compound-specific variations.

Table 1. *Results of the phytochemical composition survey of F. auriculata leaves collected in Dong Nai (DN).*

Compound Group	DN-Heptane	DN-Ethanol	Hydrolyzed DN-Ethanol	DN-Water	Hydrolyzed DN-Water	Overall Qualitative Result
Lipids	–	■	■	■	■	–
Carotenoids	–	■	■	■	■	–
Essential oils	–	■	■	■	■	–
Free triterpenoids	+	■	■	■	■	+
Alkaloids	–	–	■	–	■	–
Coumarins	–	–	–	■	■	–
Antraglycosides	–	■	–	■	+	+
Flavonoids	–	+	–	+	+	+
Cardiac glycosides	■	■	■	■	■	■
Anthocyanoids	■	+	■	–	■	+
Proanthocyanidins	■	+	■	+	■	+
Tannins	■	+	■	+	■	+
Hydrolyzed triterpenoids	■	■	+	■	+	+

Saponins	■	+	■	+	■	+
Organic acids	■	+	■	±	■	+
Reducing substances	■	++	■	+	■	++
Polyuronic compounds	■	■	■	+++	■	+++

Note: ■ = reaction present but not clearly detectable; ■ = potential reaction, but not observed; – = absent; ± = suspected; + = present; ++ = high amount; +++ = very high amount. Standard deviation (n = 3).

Table 2. *Results of the phytochemical composition survey of F. auriculata leaves collected in Dak Lak (DLK).*

Compound Group	DLK-Heptane	DLK-Ethanol	Hydrolyzed DLK-Ethanol	DLK-Water	Hydrolyzed DLK-Water	Overall Qualitative Result
Lipids	–	■	■	■	■	■
Carotenoids	±	■	■	■	■	±
Essential oils	–	■	■	■	■	■
Free triterpenoids	+	■	■	■	■	+
Alkaloids	–	–	■	–	■	–
Coumarins	–	–	–	■	■	–
Antraglycosides	±	■	±	■	±	±
Flavonoids	+	+	–	+	+	+
Cardiac glycosides	■	■	■	■	■	■
Anthocyanoids	■	+	■	±	■	+
Proanthocyanidins	■	+	■	+	■	+
Tannins	■	++	■	+	■	+
Hydrolyzed triterpenoids	■	■	+	■	+	+
Saponins	■	+	■	+	■	+
Organic acids	■	+	■	±	■	+
Reducing substances	■	++	■	+	■	+
Polyuronic compounds	■	■	■	±	■	±

Note: ■ = reaction present but not clearly detectable; ■ = potential reaction, but not observed; – = absent; ± = suspected; + = present; ++ = high amount; +++ = very high amount. Standard deviation (n = 3).

Table 3. *Results of the phytochemical composition survey of F. auriculata leaves collected in Hue (H).*

Compound Group	H-Heptane	H-Ethanol	Hydrolyzed H-Ethanol	H-Water	Hydrolyzed H-Water	Overall Qualitative Result
Lipids	–	■	■	■	■	+

Carotenoids	±	■	■	■	■	+
Essential oils	–	■	■	■	■	■
Free triterpenoids	+	■	■	■	■	■
Alkaloids	–	–	■	–	■	–
Coumarins	–	–	–	■	■	–
Antraglycosides	±	■	±	■	±	–
Flavonoids	+	+	–	+	+	+
Cardiac glycosides	■	■	■	■	■	■
Anthocyanoids	■	+	■	±	■	+
Proanthocyanidins	■	+	■	+	■	+
Tannins	■	++	■	+	■	+
Hydrolyzed triterpenoids	■	■	+	■	+	+
Saponins	■	+	■	+	■	+
Organic acids	■	+	■	±	■	+
Reducing substances	■	++	■	+	■	+
Polyuronic compounds	■	■	■	±	■	■

Note: ■ = reaction present but not clearly detectable; ■ = potential reaction, but not observed; – = absent; ± = suspected; + = present; ++ = high amount; +++ = very high amount. Each test was performed in triplicate (n = 3).

Polar solvents (ethanol and water) demonstrated superior extraction efficiency for polyphenolic compounds—particularly flavonoids, tannins, and proanthocyanidins—consistent with the 'like dissolves like' principle and with prior reports on *F. auriculata* fruit [1]. This can be explained by hydrogen bonding and dipole–dipole interactions between polar compounds and polar solvents [2]. Non-polar heptane extracts showed limited phytochemical diversity; free triterpenoids were the only consistently detected group, reflecting their hydrophobic nature [11].

Geographical origin further modulated phytochemical accumulation. Notably, polyuronic compounds were detected at very high levels (+++) in the DN-W, whereas only trace or low levels (± to –) were observed in Dak Lak and Hue samples. Environmental factors such as high temperature, seasonal drought, soil salinity, and nutrient-poor soils can stimulate the biosynthesis and accumulation of uronic acid-rich polysaccharides in plants as part of their adaptive defense mechanisms. These compounds may contribute to water retention, osmotic regulation, and protection against oxidative stress under unfavorable environmental conditions. This variation likely reflects differences in soil composition, humidity, and ecological stress among regions, which are known to influence secondary metabolite biosynthesis [1]. Additionally, alkaloids and coumarins were detectable only after hydrolysis, suggesting predominant occurrence in glycosidic (bound) forms.

The DN-W exhibited a "very high" accumulation of polyuronic compounds, indicating the plant's adaptation to specific ecological conditions in Southeastern Vietnam. In contrast, Hue's environmental factors favored the biosynthesis of complex flavonoids and catechin derivatives, resulting in a more effective phytochemical profile. Overall, the findings suggest that *F. auriculata* leaves from Central

Vietnam, extracted with polar solvents, are promising candidates for developing natural antioxidant and antimicrobial agents.

3.2. HPLC–MS–ESI Chemical Characterization

The detailed mass-to-charge *(m/z)* ratios, fragment ions, and the distribution of these metabolites across different geographical samples and solvent fractions are summarized in Table 4.

Table 4. *Tentative identification of major phytochemical compounds in Ficus auriculata leaf extracts from different Vietnamese regions using HPLC–MS-ESI.*

No.	Samples	Compound	m/z	Bioactivity
1	H-He	3-*O*-caffeoylquinic acid	352.7 (191)	Antioxidant; antimicrobial activity [12], [13]
2		4-*O*-caffeoylquinic acid	352.7 (191)	Antioxidant; antimicrobial activity [12], [13]
3		5-*O*-caffeoylquinic acid	352.7 (191, 179)	Antioxidant; antimicrobial activity [12], [13]
4		Kaempferol hexoside rhamnoside	593.8 (503, 473)	Antioxidant; anti-inflammatory activity [14]
5		3-Methyl epigallocatechin gallate	471.09 (295, 173)	Strong antioxidant; antibacterial activity [15]
6		Quercetin 3-sambubioside (Kaempferol-3-*O*-rutinoside)	594.6 (300, 271)	Antioxidant; antimicrobial activity [16]
7		Trihydroxy-octadecadienoic acid	329.3 (211)	Anti-inflammatory activity [17]
8		Hydroxy-octadecatrienoic acid	293.3 (275)	Anti-inflammatory activity [18]
9	DLK-Et	3-*O*-caffeoylquinic acid	352.7 (191)	Antioxidant; antimicrobial activity [12], [13]
10		4-*O*-caffeoylquinic acid	352.7 (191)	Antioxidant; antimicrobial activity [12], [13]
11		5-*O*-caffeoylquinic acid	352.7 (191, 179)	Antioxidant; antimicrobial activity [12], [13]
12		Kaempferol hexoside rhamnoside	593.8 (503, 473)	Antioxidant; anti-inflammatory activity [14]
13		3-Methyl epigallocatechin gallate	471.09 (295, 173)	Strong antioxidant; antibacterial activity [15]
14		Quercetin 3-sambubioside	594.6 (300, 271)	Antioxidant; antimicrobial activity [16]
15		Trihydroxy-octadecadienoic acid	329.3 (211)	Anti-inflammatory activity; lipid bioactivity [17]
16		Hydroxy-octadecatrienoic acid	293.3 (275)	Anti-inflammatory activity [18]
17	H-Et	Epicatechin (catechin)	289.1-288.9 (245, 179)	Antioxidant [19]
18		Catechin glucoside	448.8 (287, 125)	Antioxidant activity [20]

19		Apigenin 8-*C*-glucoside	576.6 (401, 413)	Antioxidant; anti-inflammatory activity [21]
20	H-W	Hydroxybenzoic acids I and II	137.03; 67.04	Antioxidant; antimicrobial activity [22]
21		Coumaroylmalic acid	279.05; 193.05	Antioxidant; antimicrobial activity [23]
22		Naringenin, eriodictyol	271.06; 287.06	Antioxidant; anti-inflammatory activity [24]
23		Genistein	269.051; 353.10	Antioxidant; anti-inflammatory; estrogenic activity [25]
24		Prenylhydroxygenistein	337.10; 353.10	Antioxidant; anti-inflammatory; antimicrobial activity [26]
25		(2Z)-3-[6-(*β*-D-glucopyranosyloxy)-1-benzofuranyl]-2-propenoic acid (psoralic acid glucoside)	339.07; 365.09	Antioxidant; antimicrobial activity [27]
26		Hydroxypsoralen hexoside Iia	365.08	Antioxidant; photobiological activity [28]
27		Marmesin isomer	247.09	Anti-inflammatory; antimicrobial activity [29]
28		Psoralic acid	205.05	Antimicrobial; antifungal; photobiological activity [30]
29	DLK-He* DLK-W* DN-W* DN-Et* DN-He*	Not detected	-	-

Abbreviations: DLK = Dak Lak; H = Hue; DN = Dong Nai; He = Heptane; W = Water; Et = Ethanol.

Note: Each test was performed in triplicate ($n = 3$).

The detailed mass-to-charge (*m/z*) ratios, fragment ions, and the distribution of these metabolites across different geographical samples and solvent fractions are summarized in Table 4. A total of 28 compounds were tentatively identified in the Hue and Dak Lak extracts, whereas no compounds were tentatively identified in any of the Dong Nai extracts, including DN-He, DN-Et, and DN-W, under the analytical conditions employed in this study.

The HPLC–MS–ESI analysis of *Ficus auriculata* leaf extracts revealed significant variations in chemical composition based on solvent polarity and geographical origin. Polar fractions (ethanol and distilled water) contained a greater diversity of polyphenolic and flavonoid compounds, while heptane fractions primarily held non-polar terpenoids.

Key bioactivity markers included phenolic acid derivatives, particularly isomers of caffeoylquinic acid (*m/z* = 352.7), and flavonoid glycosides such as quercetin 3-sambubioside. Notably, 3-methyl epigallocatechin gallate (*m/z* = 471.09) and catechin glucoside (*m/z* = 448.8) found in the H-Et correlated with superior antioxidant performance in DPPH and FRAP assays.

3.3. Antioxidant Activities

The antioxidant activities of all extracts, expressed as IC_{50} values from DPPH and FRAP assays, are presented in Figure 1. Polar extracts consistently outperformed heptane fraction across both assays and all regions.

In the DPPH assay (Figure 1), the H-W exhibited the strongest radical scavenging activity (IC_{50} = 11.28 ± 0.22 µg/mL), followed by the H-Et (13.81 ± 0.26 µg/mL) and DN-W (14.57 ± 0.21 µg/mL). Heptane extracts were markedly weaker (IC_{50} > 186 µg/mL) or undetectable (DLK-He). The FRAP

assay mirrored this trend: H-W (EC_{50} = 4.89 ± 0.08 µg/mL) > DN-W > ethanol extracts > heptane fractions. These results are in good agreement with the phytochemical profile in Section 3.1; the abundance of flavonoids, tannins, and proanthocyanidins in polar fractions directly accounts for their capacity to donate hydrogen atoms (DPPH assay) and reduce Fe^{3+} to Fe^{2+} (FRAP assay) [31], [32].

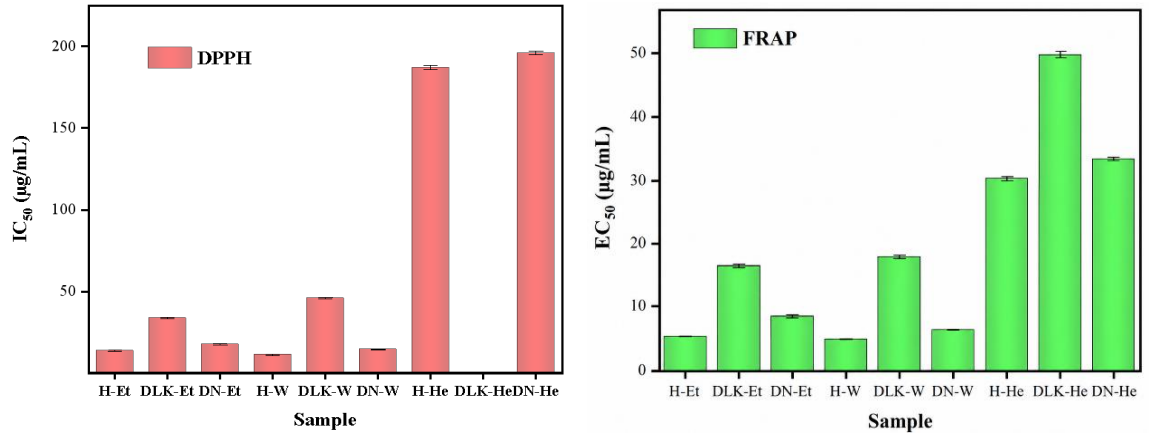

***Figure 1**. Antioxidant activities of Ficus auriculata leaf extracts expressed as IC_{50} values (µg/mL) in the DPPH assay (A) and EC_{50} values (µg/mL) FRAP assay (B).*

Geographical variation significantly influenced antioxidant capacity. Hue extracts were consistently superior, followed by Dong Nai, while Dak Lak extracts exhibited the lowest activity. This gradient corresponds to differences in polyphenol accumulation observed during phytochemical screening and may reflect the cooler, more humid climate of Hue, promoting secondary metabolite biosynthesis. Strong correlations between total phenolic content and antioxidant capacity in *Ficus* species have been documented [1].

4. Conclusions

This study demonstrated that solvent polarity is the primary determinant of phytochemical diversity and antioxidant activity in *Ficus auriculata* leaf extracts from three Vietnamese regions (Hue, Dak Lak, and Dong Nai), while geographical origin introduced compound-specific secondary modulation. Polar solvents (ethanol and distilled water) yielded markedly richer polyphenolic profiles–including flavonoids, tannins, proanthocyanidins, and reducing substances–compared to non-polar heptane, which was limited to free triterpenoids. Alkaloids and coumarins were detectable only in hydrolyzed fractions, indicating their predominant occurrence in glycosidic forms. HPLC–MS–ESI analysis identified key bioactive constituents in polar fractions, including caffeoylquinic acid isomers, flavonoid glycosides, catechin derivatives, and coumarins, with the DN-W uniquely accumulating high levels of polyuronic compounds reflecting adaptation to southeastern ecological conditions. DPPH and FRAP assays confirmed the superior antioxidant capacity of polar extracts across all origins; the H-W showed the strongest activity (DPPH IC_{50} = 11.28 ± 0.22 µg/mL; FRAP EC_{50} = 4.89 ± 0.08 µg/mL), consistent with its enriched flavonoid and catechin content, and suggesting that the cooler, more humid climate of Central Vietnam promotes enhanced polyphenol accumulation. These results provide a scientific basis for developing *F. auriculata*-derived natural antioxidant agents, with polar extracts from Hue province as the most promising candidates. Future work should focus on quantitative compound profiling, synergistic activity evaluation, and *in vivo* validation to support biomedical applications.

Acknowledgments

We would like to thank Ho Chi Minh City University of Technology and Engineering for the support.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, AI-assisted tools were used for language editing. The authors reviewed and edited all content and take full responsibility for the publication.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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