ISSN 1859 - 4735

JOURNAL OF MEDICINAL MATERIALS

No. 5 - Vol. 30
9/2025

NATIONAL INSTITUTE OF MEDICINAL MATERIALS

3B QUANG TRUNG - CUA NAM - HANOI - VIETNAM
TEL: (+84-24) 38247058 FAX: (+84-24) 39348740
Email: tapchiduoclieu@nimm.org.vn; tapchiduoclieu@gmail.com

JOURNAL OF MEDICINAL MATERIALS (Print and Electronic Journals)

EDITORIAL BOARD (Term 2025-2030)

(Attached to Decision No. 666/QD-VDL dated April 24, 2025 signed by the Director of Vietnam National Institute of Medicinal Materials)

EDITOR-IN-CHIEF

Assoc. Prof. PhD. Do Thi Ha

I. Subcommittee of Pharmacognosy - Traditional Medicine

- **Assoc. Prof. Dr. Sci. Nguyen Minh Khoi - Head of Subcommittee**
- Assoc. Prof. PhD. Nguyen Thuong Dong - Member
- Prof. PhD. Nguyen Minh Duc - Member
- Prof. PhD. Tran Cong Luan - Member
- Prof. PhD. Won Keun Oh - Member
- Assoc. Prof. PhD. Tran Hung - Member
- Assoc. Prof. PhD. Nguyen Thi Bich Thu - Member
- Assoc. Prof. PhD. Phuong Thien Thuong - Member
- Assoc. Prof. PhD. Phan Minh Giang - Member
- Assoc. Prof. PhD. Le Nguyen Thanh - Member
- Assoc. Prof. PhD. Le Viet Dung - Member
- Assoc. Prof. PhD. Nguyen Quoc Huy - Member
- Assoc. Prof. PhD. Nguyen Thu Hang - Member
- PhD. Tran Minh Ngoc - Member
- PhD. Nguyen Van Tai - Member
- Assoc. Prof. PhD. Le Dinh Chi - Member
- PhD. Nguyen Tuan Hiep - Member.

II. Subcommittee of Pharmacology - Clinical Pharmacy

- **Assoc. Prof. PhD. Pham Thi Nguyet Hang - Head of Subcommittee**
- Assoc. Prof. Dr.Sci. Do Trung Dam - Member
- Assoc. Prof. PhD. Nguyen Thi Thu Huong - Member
- Assoc. PhD. William R. Folk - Member
- Assoc. Prof. PhD. Pham Thi Van Anh - Member
- Assoc. Prof. PhD. Nguyen Thuy Duong - Member
- Assoc. Prof. PhD. Le Van Minh - Member
- PhD. Tran Thi Hien - Member.

III. Subcommittee of Agriculture - Biology

- **PhD. Nguyen Van Khiem - Head of Subcommittee**
- PhD. Phan Thuy Hien - Member
- Assoc. Prof. PhD. Nguyen Van Tap - Member
- Assoc. Prof. PhD. Pham Thanh Huyen - Member
- Prof. PhD. Tran The Bach - Member.

IV. Subcommittee of Secretariat Administration

- **Assoc. Prof. PhD. Le Nguyen Thanh - Head of Subcommittee**
- PhD. Le Thi Xoan - Academic Secretary
- BSc. Nguyen Thi Minh Loc - Administrative Secretary.

CONTENTS VOL. 30(5), 2025
(Pages 305 - 384)

Page	SCIENTIFIC RESEARCH
307	**Acetylcholinesterase Inhibitory Activity of Triterpenoids and Alkaloids Isolated from *Lycopodiella cernua* (L.) Pic. Serm. -** *Le Ba Vinh, Nguyen Thi Nga, Nguyen Cao Cuong, Nguyen Van Chinh, Nguyen Ngoc Linh*
313	**Chemical Constituents from the Seeds of *Ziziphus mauritiana* Lam. Collected in Vietnam -** *Do Hoang Anh, Nguyen Tra My, Le Hong Van Anh, Nguyen Thi Thu, Tran Huyen Trang, Phan Van Truong, Do Thi Ha, Le Nguyen Thanh, Nguyen Thi Hang, Le Thanh Nghi*
320	**Antimicrobial Diketopiperazine Alkaloids and Indole Derivatives from the Marine-Derived Fungus *Penicillium* sp. M874 -** *Phi Thi Dao, Nguyen Thuy Linh, Le Thi Hong Minh, Doan Thi Mai Huong, Pham Van Cuong*
327	**Alkaloids Isolated from *Crinum viviparum* (Lam.) R.Ansari & V.I.Nair and their Biological Activities -** *Vu Thi Trang, Ngo Viet Duc, Pham Van Cong, Ngo Van Hieu, Nguyen Tien Dat, Do Thanh Tuan, Dang Viet Hau, Nguyen Thi Thanh, Hoang Le Tuan Anh, Le Quynh Lien*
333	**Chemical Constituents and Antimicrobial Activity of the Marine Sediment-Derived Fungus *Aspergillus* sp. M793 -** *Nguyen Thuy Linh, Phi Thi Dao, Le Thi Hong Minh, Pham Van Cuong, Doan Thi Mai Huong*
341	**Lignans and Neolignan from the Stems of *Cansjera rheedei* J.F.Gmel. -** *Tran Duy Hien, Vo Thanh Hoa, Bui Nguyen Bien Thuy*
346	**Aryltetralin Lignans, Phenolic Acid Derivatives, and an Alkyl Glycoside from Rhizomes of *Dysosma tonkinense* -** *Nguyen Cong Luong, Nguyen Thi Thu, Do Hoang Anh, Le Hong Van Anh, Tran Minh Ngoc, Do Thi Ha*
353	**Chemical Composition and *In Vitro*/*In Silico* Enzyme Inhibitory Activities of the Leaf Essential Oil of *Actinodaphne pilosa* (Lour.) Merr. from Quang Tri Province -** *Le Duc Anh Minh, Ngu Thi Tra Giang, Dang Su Uyen, Doan Thi Huyen Linh, Tran Gia Nhu, Hoang Van Luu*
359	**Simultaneous Determination of Chlorogenic Acid and Isochlorogenic Acid A in *Pharbitidis Semen* by HPLC Method -** *Pham Thi Hien, Nguyen Thi Ha Ly, Nguyen Thi Kim Oanh, Nguyen Manh Khoa, Nguyen Huy Van, Tran Quang Luc, Vu Huong Thuy, Nguyen Thi Van Anh, Nguyen Thi Vinh Hue, Do Thi Ha*
368	**Microscopic Characteristics, HPTLC Profiling, and Evaluation of Antioxidant, Tyrosinase Inhibitory, and Anti-Inflammatory Activities of *Eurya nitida* Korth. Leaf Extracts -** *Nguyen Phuc Linh Gabriel, Nguyen Minh Tien, Ngo The Kieu Trang, Nguyen Ngoc Hoang Nhi, Huynh Ha Thy, Nguyen Phuc Khanh, Vu Huynh Kim Long, Nguyen Minh Hien*
375	**Standardized Extracts of *Paris polyphylla* var. *chinensis* Smith Rhizomes and *Piper longum* L. Fruits Suppress Breast Cancer Cell Migration -** *Hoang Thi Phuong Thao, Ly Hai Trieu, Nguyen Thi Thu, Do Thi Ha, Le Van Minh*

Journal of Medicinal Materials, 2025, Vol. 30, No. 5 (368 - 374)

MICROSCOPIC CHARACTERISTICS, HPTLC PROFILING, AND EVALUATION OF ANTIOXIDANT, TYROSINASE INHIBITORY, AND ANTI-INFLAMMATORY ACTIVITIES OF *EURYA NITIDA* KORTH. LEAF EXTRACTS

Nguyen Phuc Linh Gabriel[1], Nguyen Minh Tien[1], Ngo The Kieu Trang[1], Nguyen Ngoc Hoang Nhi[1], Huynh Ha Thy[1], Nguyen Phuc Khanh[1], Vu Huynh Kim Long[2], Nguyen Minh Hien[2,*]

[1]Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam;
[2]Research Group in Pharmaceutical and Biomedical Sciences, Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam
*Corresponding author: nguyenminhhien@tdtu.edu.vn
Received July 14th, 2025 Accepted August 18th, 2025

Summary

The microscopic study, high performance thin layer chromatography (HPTLC) profile, antioxidant, tyrosinase inhibitory properties, and anti-inflammatory properties of *n*-hexane, ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), water, and methanol (MeOH) extracts of *Eurya nitida* Korth. leaf were investigated for the first time. Besides total phenolic content (TPC) and total flavonoid content (TFC), the antioxidant determinations encompassed 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), and reducing power (RP). EtOAc extract exhibited the most potent radical scavenging capacities for DPPH (IC_{50} = 11.10 ± 0.18 µg/mL), ABTS (IC_{50} 7.63 ± 0.04 µg/mL), RP ($OD_{0.5}$ 78.65 ± 0.63 µg/mL), FRAP (320.31 ± 14.34 mg AAE/g), TAC (123.2 ± 3.88 mg AAE/g), as well as significantly high TPC (292.41 ± 2.47 mg GAE/g) and TFC (76.30 ± 9.87 mg QE/g). Moreover, the EtOAc extract was found to possess the strongest anti-tyrosinase activity (37.96 ± 0.80% inhibition at 1000 µg/mL) among all tested samples. In addition, anti-inflammatory properties were also evaluated. Among all extracts, the EtOAc extract showed the highest inhibition of nitric oxide (NO) production in LPS-activated RAW 264.7 macrophages (IC_{50} 25.62 ± 1.38 µg/mL), without showing cytotoxicity. In conclusion, *Eurya nitida* Korth. leaf presents a promising source of natural products for therapeutic applications in mitigating oxidative stress and anti-inflammation.

Keywords: *Antioxidant; Tyrosinase inhibition; Anti-inflammation; HPTLC fingerprinting analysis; Microscopic characteristics; Eurya nitida Korth. leaf extracts.*

1. Background

Eurya nitida Korth. is a woody shrub, 1-6 meters tall, with dark brown branches, light purple spherical berries, and clusters of three white flowers in the leaf axils, emitting a pungent, unpleasant odour. Across humid temperate tropical regions, especially the mountains of Northern Vietnam, it is known as Súm or Chè cẩu. Traditionally, its leaves are used to alleviate dysuria, nocturia, and hematuria. Beverages are made from young leaves, buds, and flowers. Meanwhile, the fruit and seeds are employed for dye production [1]. Moreover, a recent study reporting a high polyphenolic content in its fruit has highlighted its potential for antioxidant and tyrosinase inhibitory activity assessments [2]. Despite its traditional ethnomedical applications, no scientific investigations have been conducted on the phytochemical composition and pharmacological properties of *E. nitida* Korth. leaves. Continuing the search for bioactive extracts, this study investigated the microscopic characteristics of *E. nitida* Korth. leaf powder and transverse leaf sections, as well as the antioxidant and tyrosinase inhibitory activities of its extracts. Furthermore, the investigation was expanded to assess its anti-inflammatory efficacy. These findings support the development of natural therapeutics for managing oxidative stress and inflammation.

2. Materials and methods

2.1. Plant material and extraction

Fresh *E. nitida* Korth. leaves were sampled at Cam Nghia commune, Cam Lo district, Quang Tri province, Vietnam, and authenticated by Dr. Le Tuan Anh (Central Institute for Biological Research, Vietnam Academy of Science and Technology). The dried *Eurya nitida* Korth. leaves (1 kg) were processed through maceration with MeOH (5 L × 3) every three days to obtain crude extract (47.50 g), and through solvent-solvent partitioning (2 L × 3, each solvent) to produce *n*-hexane (12.10 g), EtOAc (8.84 g), *n*-BuOH (7.12 g), and water extract (5.01 g). These

extracts were diluted to various concentrations for bioactivity assays.

2.2. General procedures

A Thermo Fisher Scientific UV-Vis spectrophotometer was used for measuring the absorbance in antioxidant tests. Meanwhile, an ELISA Plate Reader (Bio-Rad) was used for anti-tyrosinase and inhibition of NO production. Chemicals purchased from Sigma-Aldrich (USA) include enzyme tyrosinase, dimethyl sulfoxide (DMSO), vitamin C (BCCH6669; 99.0%), quercetin (10230364; 97.0%), DPPH, ABTS, Folin-Ciocalteu (FC) reagent, TPTZ, LPS, and *L*-NMMA. Gallic acid (5995-86-8; 98.0%) was obtained from HiMedia.

2.3. Microscopic study

The *E. nitida* leaves were cut and bleached with Javelle's solution for color removal, and then thoroughly cleaned and immersed in acetic acid before being stained with the alum carmine-methyl green reagent. Stained samples were washed with water to eliminate any excess reagent before inspection under a light microscope. Additionally, the leaf specimens were mashed with a stainless steel mesh after being dried at 60°C for powder microscopy, following the Khandelwal method [3].

2.4. HPTLC fingerprinting assay

HPTLC fingerprinting studies were performed according to K. Karthika and S. Paulsamy's method [4]. A 20 mg MeOH leaf extract was dissolved in 0.5 mL of MeOH and centrifuged at 8000 rpm for 5 minutes. Using a CAMAG HPTLC system with LINOMAT 5, 2 µL of the supernatant was applied to a *silica gel* 60 F_{254} TLC plate (5 × 10 cm). Plates were developed in a twin trough chamber with mobile phases specific to alkaloids, anthracenes, cardiac glycosides, essential oils, saponins, triterpenes, and valepotriates, then heated at 110°C. Images were captured under visible light and UV at 254 nm and 366 nm using a photo-documentation chamber.

2.5. Antioxidant assays of the extracts

2.5.1. Total phenolic content (TPC) and Total flavonoid content (TFC):

Based on Nguyen et al. (2023) and Chang et al. (2020) with minor modifications, the calibration curves were constructed based on gallic acid for TPC from the stock 5 mg/500 µL in DMSO, and diluted with water to obtain the final concentrations (25; 12.5; 6.25; 3.125; 1.5625 µg/mL). Quercetin is used as the standard compound for TFC from the stock 5 mg/500 µL and diluted with water to obtain the final concentrations (125; 62.5; 31.25; 15.625; 7.8125; 3.906 µg/mL), using the FC (λ = 760 nm) and $AlCl_3.6H_2O$ 0.2 M/CH_3COOK 1 M methods (λ = 415 nm), respectively [5],[6]. The extracts were prepared at 50 µg/mL for the TPC assay and 1 mg/mL for the TFC assay.

2.5.2. DPPH and ABTS radical scavenging assays:

Following Baliyan et al. (2022) and Re et al. (1999) with slight adjustments, DPPH and ABTS radical scavenging activities of the samples were assessed colorimetrically at λ = 517 nm and λ = 734 nm, respectively [7],[8].

2.5.3. Ferric reducing antioxidant power (FRAP), reducing power (RP), and total antioxidant capacity (TAC) assays:

With insignificant changes to the methods of Benzie and Strain (1996), Oyaizu (1986), and Tsao et al. (2024), five extracts were evaluated for FRAP, RP, and TAC, respectively. FRAP was measured at λ = 593 nm using 10 mM TPTZ, 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ as reagents; RP at λ = 700 nm using $K_3[Fe(CN)_6]$ 1%, CCl_3COOH 10%, and $FeCl_3$ 0.1%; and TAC at λ = 570 nm using molybdate [9],[10],[11].

2.6. Anti-tyrosinase activity

Following Chen et al.'s procedure, the experiment was carried out with minor adjustments. In each of the 96-well microplates, 100 µL of enzyme tyrosinase solution (100 U/mL) was mixed with 40 µL of the extract or positive control solution and incubated in the dark for 10 minutes at room temperature. Then, 60 µL of *L*-3,4-dihydroxyphenylalanine (*L*-DOPA) solution (3 mM) was added to each well and incubated for 8 minutes at 37°C. The absorbance of the sample was measured at λ = 490 nm. Vitamin C served as a positive control for this experiment [12].

2.7 Anti-inflammatory activity: Nitric oxide (NO) inhibition in RAW 264.7 cells activated by Lipopolysaccharide (LPS)

The anti-inflammatory efficacy was assessed via NO inhibition assay using the Griess reaction, with modifications based on Adebayo et al. (2015). RAW 264.7 macrophages were seeded at 4×10^4 cells/well and incubated for 24 hours. Test extracts, diluted in Dulbecco's Modified Eagle Medium (DMEM) and pre-incubated with the cells for 2 hours, followed by stimulation with LPS (1 µg/mL) for an additional 24 hours. The supernatant was transferred to a new 96-well microplate, and 100 µL of Griess reagent was

added, with incubation for 15 minutes. NO levels in the culture supernatant were measured at λ = 540 nm, with N^G-monomethyl-*L*-arginine acetate (*L*-NMMA) was used as the positive control [13]. The remaining cell layer was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to determine cell viability [14].

2.8. Statistical analysis

Data are expressed as mean ± standard deviation (n=3). GraphPad Prism 9.3.1 (San Diego, CA, USA) was applied for statistical data.

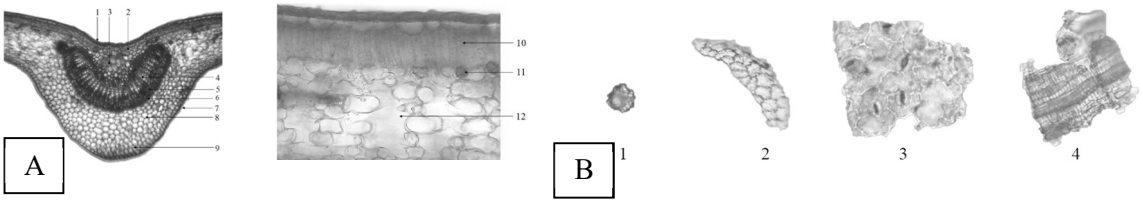
3. Results

3.1. Transverse section analysis and powder microscopic investigation of the leaf

Transverse section of the leaf midrib: The upper epidermis is a single layer of rectangular cells with a cuticle. Upper and lower collenchyma are composed of two thick-walled polygonal cells. Upper parenchyma contains three to four lignin-wall cells, while the lower parenchyma consists of many layers of cellulose-wall polygonal cells. Fibers' sclerenchyma forms a two-layered sheath of thick-walled cells around the main bundle. Vascular bundles are arc-shaped, with xylem above and phloem below. Xylem vessels are circular polygons arranged in 33 radial rows, each with 3-5 xylem cells. Phloem clusters contain curved polygonal cells. Spherical calcium oxalate crystals are abundant in the parenchyma and phloem.

Stomata are frequent in the lower epidermis. The palisade mesophyll is a layer of rectangular cells. The spongy mesophyll has roughly round polygonal cells. Spherical calcium oxalate crystals are scattered in the palisade mesophyll (Fig. 1A).

Leaf powder appears dark green with a slightly bitter taste. Many pieces of epidermis, including the lower epidermis with stomata, bordered pitted vessels, and calcium oxalate crystals, were observed in the leaves (Fig. 1B).



1. Upper epidermis, 2. Upper collenchyma, 3. Parenchyma with lignified wall, 4. Xylem, 5. Phloem, 6. Sclerenchyma, 7. Lower epidermis, 8. Lower parenchyma, 9. Lower collenchyma

1. Calcium oxalate crystal, 2. Epidermis, 3. Lower epidermis with stomata, 4. Bordered pitted vessels

Fig. 1. The transverse section of the leaf (x10) and Microscopic Features of *E. nitida* leaf. (A) The transverse section of the leaf x10), (B) Microscopic features (x40)

3.2. HPTLC fingerprinting analysis

Preliminary phytochemical analysis indicates the presence or absence of compounds in extracts from plants based on visual inspection of color. The HPTLC fingerprinting for methanolic leaf extracts of *E. nitida* exposed the existence of essential oils, bitter compounds, triterpenes, saponins, valepotriates, alkaloids, cardiac glycosides, flavonoids, and lignans (Fig. 2 and Table 1).

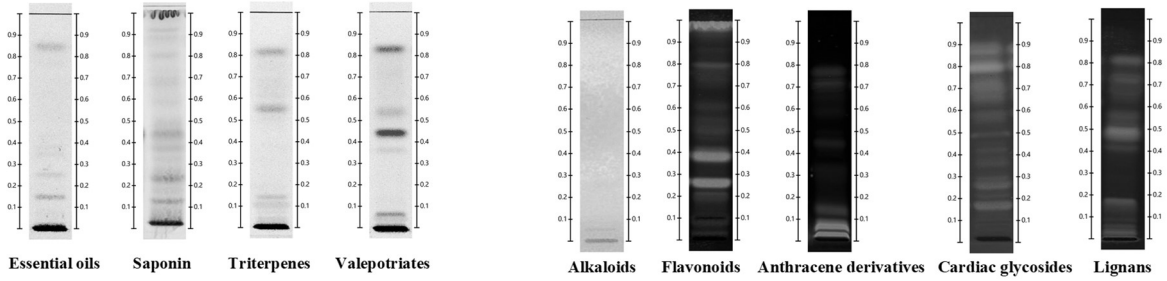


Fig. 2. HPTLC chromatograms of phytoconstituents for *Eurya nitida* Korth. leaf extracts

Table 1. Mobile phase, spray reagent used, and the changed color for respective compounds

Group compounds	Mobile phase (*v/v* or *v/v/v* or *v/v/v/v*)	Spray reagents	Observation	
			Visible light	UV 366 nm
Alkaloids	Toluene:ethyl acetate:diethylamine (70:20:10)	Dragendorff's reagent (Bismuth nitrate 0.34%, potassium iodide 8%, *w/v*, in 4% acetic acid aqueous)	Yellow, orange-yellow	–
Anthracene derivatives	Ethyl acetate:methanol:water (100:13.5:10)	Potassium hydroxide (5% *w/v* in 96% methanol)	–	Yellow, red-brown
Essential oils	Toluene:ethyl acetate (93:7)	Anisaldehyde sulphuric acid (methanol:acetic acid:sulphuric acid:*p*-anisaldehyde 85:10:5:0.5, *v/v/v/v*)	Blue, green, red, and brown	–
Saponin	Chloroform:glacial acetic acid: methanol:water (6.4:3.2:1.2:0.8)		Blue, Blue-violet, red and yellow-brown	–
Triterpenes	*n*-hexane:ethyl acetate (1:1)		Blue-violet, red, and red-violet	–
Valepotriates	Toluene:ethyl acetate (75:25)		Violet or blue	–
Flavonoids	Ethyl acetate:water:formic acid:glacial acetic acid (100:26:11:11)	Sulphuric acid (10% *v/v* in methanol)	–	Blue, green, red
Cardiac glycosides	Ethyl acetate:methanol:water (81:11:8)		–	Blue, Brown, green, and yellowish
Lignans	Toluene:ethyl acetate (7:3)		–	Blue

3.3. Antioxidant activity of the extracts

3.3.1. TPC and TFC assays:

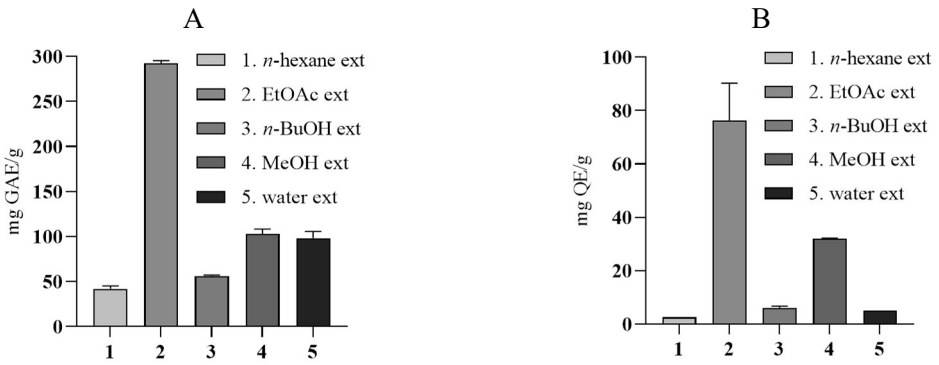


Figure 3. TPC (A) and TFC (B) of the extracts

The quantitative values were expressed as mg of positive control equivalent per gram of dried extracts. For TPC, gallic acid at concentrations of 25, 12.5, 6.25, 3.125, and 1.5625 µg/mL was used as the standard, showing corresponding absorbance values of 1.798, 0.974, 0.558, 0.280, and 0.164, respectively. A calibration curve based on gallic acid concentrations ($y = 0.0693x + 0.0833$, $R^2 = 0.9978$) was used to calculate TPC via the Folin-Ciocalteu method. The highest TPC was found in the EtOAc extract (292.41 ± 2.47 mg GAE/g), followed by MeOH (104.64 ± 4.20), water (98.18 ± 6.01), *n*-BuOH (56.77 ± 0.58), and *n*-hexane (41.76 ± 2.83) extracts (Fig. 3A, Table 2). Similarly, for TFC, standard solutions of quercetin (125, 62.5, 31.25, 15.625, 7.8125, and 3.906 µg/mL) were prepared, and their absorbances were measured as 0.902, 0.450, 0.223, 0.121, 0.062, and 0.039, respectively. Its regression equation for TFC calculation was $y = 0.0071x + 0.0063$, $R^2 = 0.9998$. As a result, the EtOAc extract exhibited the highest TFC (76.30

± 9.87 mg QE/g), followed by MeOH (31.98 ± 0.14), *n*-BuOH (6.01 ± 0.49), water (5.10 ± 0.01), and *n*-hexane (2.58 ± 0.01) extracts (Fig. 3B, Table 2).

3.3.2. DPPH and ABTS assays

Compared to the positive control vitamin C (IC_{50} 1.60 ± 0.01 µg/mL), the EtOAc extract exhibited the strongest DPPH radical scavenging activity among the samples (IC_{50} 11.10 ± 0.18 µg/mL), followed by water (IC_{50} 48.42 ± 0.13 µg/mL), MeOH (IC_{50} 50.16 ± 0.33 µg/mL), and *n*-BuOH (IC_{50} 54.17 ± 0.63 µg/mL). The *n*-hexane extract showed negligible activity with IC_{50} > 100 µg/mL (Fig. 4A, Table 2). Similarly, in increasing order of ABTS inhibitory capacity, *n*-hexane (IC_{50} 35.10 ± 0.73 µg/mL), *n*-BuOH (IC_{50} 26.97 ± 0.11 µg/mL), MeOH (IC_{50} 21.28 ± 0.14 µg/mL), water (IC_{50} 18.87 ± 0.17 µg/mL), and EtOAc extract, which demonstrated the highest activity (IC_{50} 7.63 ± 0.04 µg/mL), while vitamin C (IC_{50} 1.73 ± 0.020 µg/mL) (Fig. 4B, Table 2).

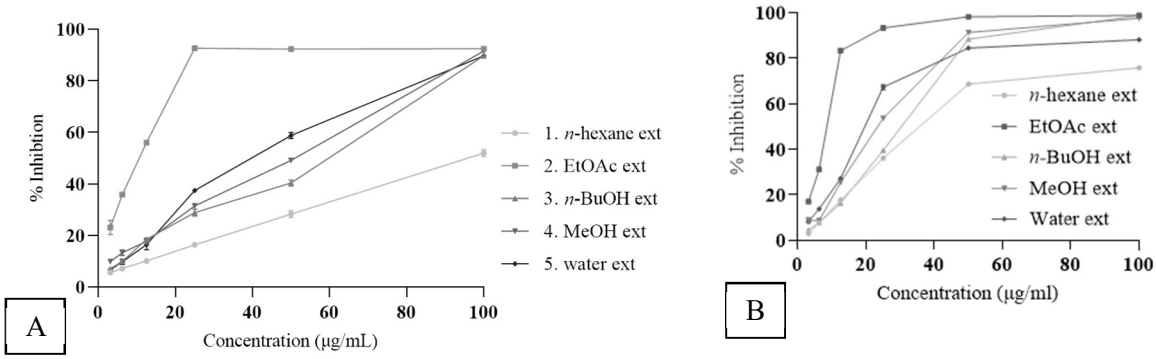


Fig. 4. DPPH (A) and ABTS (B) assays of the extracts

3.3.3. FRAP, RP, and TAC assays:

The standard curve (y = 0.0126x + 0.2055; R^2 = 0.9948) based on vitamin C, was used to determine FRAP values. The EtOAc extract exhibited the highest ferric reducing power (320.31 ± 14.34 mg AAE/g), followed by water (104.03 ± 14.62 mg AAE/g), MeOH (94.43 ± 5.38 mg AAE/g), *n*-BuOH (40.49 ± 2.06 mg AAE/g), and *n*-hexane (6.17 ± 0.02 mg AAE/g) (Fig. 5A, Table 2). RP results also varied significantly. The EtOAc extract demonstrated the strongest reducing power ($OD_{0.5}$ 78.65 ± 0.63 µg/mL), while *n*-hexane and water extracts required concentrations above 300 µg/mL. MeOH and *n*-BuOH extracts showed comparable activity (Fig. 5B, Table 2). TAC was evaluated via the reduction of Mo(VI) to Mo(V), forming a green phosphate/Mo(V) complex. The EtOAc extract again showed the highest antioxidant capacity (123.2 ± 3.88 mg AAE/g), whereas the water extract exhibited substantially lower activity (63.12 ± 1.36 mg AAE/g) (Fig. 5C, Table 2).

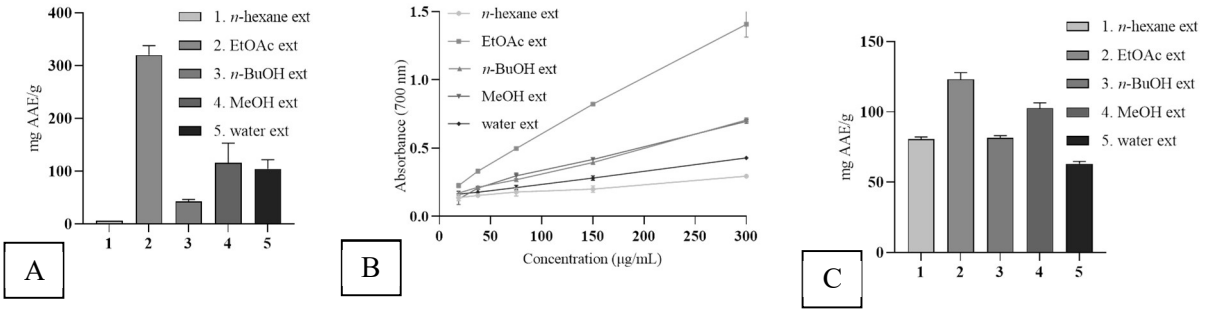


Fig. 5. FRAP (A), RP (B), and TAC (C) values of the extracts

Table 2. The TPC, TFC, DPPH, ABTS, FRAP, TAC, and RP assays of the extracts
Data are presented as the mean ± SD (n=3). * Positive controls (1.0 µg/mL ≈ 5.677 µM)

Samples	TPC ± SD (mg GAE/g)	TFC ± SD (mg QE/g)	DPPH ± SD (IC_{50} µg/mL)	ABTS ± SD (IC_{50} µg/mL)	FRAP ± SD (mg AAE/g)	TAC ± SD (mg AAE/g)	RP ± SD (OD 0.5 µg/mL)
n-hexane	41.76 ± 2.83	2.58 ± 0.01	>100	35.10 ± 0.73	6.17 ± 0.02	80.74 ± 1.18	>300
EtOAc	292.41±2.47	76.30 ± 9.87	11.10 ± 0.18	7.63 ± 0.04	320.31±14.34	123.2 ± 3.88	78.65 ± 0.63
n-BuOH	56.77 ± 0.58	6.01 ± 0.49	54.17 ± 0.63	26.97 ± 0.11	40.49 ± 2.06	81.55 ± 1.26	194.33 ± 2.65
MeOH	104.64± 4.20	31.98 ± 0.14	50.16 ± 0.33	21.28 ± 0.14	94.43 ± 5.38	102.66±3.09	195.63 ± 3.36
Water	98.18 ± 6.01	5.10 ± 0.01	48.42 ± 0.13	18.87 ± 0.17	104.03±14.62	63.12 ± 1.36	>300
Vitamin C*			1.60 ± 0.01	1.73 ± 0.020			
Quercetin*							16.56 ± 0.05

3.4. Anti-tyrosinase activity

All the extracts exhibited weak tyrosinase inhibitory effects. EtOAc extract displayed the highest inhibition at 37.96 ± 0.80% (1000 µg/mL) and 31.95 ± 1.20% (500 µg/mL) (Table 3).

Table 3. % Inhibition of tyrosinase activity.
*Data are presented as the mean ± SD (n=3); *Positive control (µg/mL)*

Extracts	% Inhibition at 1000 µg/mL	% Inhibition at 500 µg/mL
n-hexane	14.71 ± 1.43	5.29 ± 0.98
EtOAc	37.96 ± 0.80	31.95 ± 1.20
n-BuOH	19.79 ± 1.58	8.89 ± 0.69
MeOH	25.19 ± 1.56	16.83 ± 1.19
Water	9.84 ± 0.93	3.49 ± 0.52
Vitamin C*	61.06 ± 1.22	45.61 ± 1.76

3.5. Anti-inflammatory activity: Nitric oxide (NO) inhibition in RAW 264.7 cells activated by Lipopolysaccharide (LPS)

The EtOAc extract showed the most potent anti-inflammatory activity (IC_{50} 25.62 ± 1.38 µg/mL), followed by *n*-BuOH (95.92 ± 3.76 µg/mL), *n*-hexane (96.57 ± 2.35 µg/mL), and MeOH (97.25 ± 1.42 µg/mL). The water extract showed negligible NO inhibitory capacity, with an IC_{50} > 100 µg/mL. The extracts exhibited no cytotoxicity against RAW 264.7 cells at the highest concentration (100 µg/mL), as determined by the MTT assay (Table 4).

Table 4. IC_{50} values for anti-inflammatory activity in RAW 264.7 cells
*Data are presented as the mean ± SD (n=3). *Positive control (µM)*

Extracts	IC_{50} (µg/mL)	Cell viability (%) at 100 µg/mL
n-hexane	96.57 ± 2.35	97.0 ± 0.91
EtOAc	25.62 ± 1.38	100.01 ± 1.4
n-BuOH	95.92 ± 3.76	99.9 ± 0.56
MeOH	97.25 ± 1.42	94.15 ± 0.39
Water	> 100	87.88 ± 0.14
L-NMMA*	66.03 ± 2.85	98.34 ± 0.46

4. Discussion

Transverse section and powder microscopic analysis of the leaf are vital for the accurate identification and authentication of medicinal plants, revealing key diagnostic features such as calcium oxalate crystals, vascular structures, and stomatal distribution. Following this, phytochemical screening of *E. nitida* leaves confirmed the presence of various secondary metabolites, with a notable abundance of flavonoids and phenolic compounds. These compounds are primarily responsible for the plant's antioxidant activity, as reflected by TPC and TFC. The redox properties of these phytochemicals enable them to scavenge free radicals effectively, which was further validated

through antioxidant assays, including DPPH, ABTS, RP, FRAP, and TAC. Vitamin C had a tyrosinase inhibition rate of 61.06 ± 1.22% at 1000 µg/mL. This finding aligns with a previously published IC_{50} value of 960 µg/mL [12]. Although the EtOAc extract showed the highest activity among those tested, its inhibitory effect was still weaker compared to that of vitamin C. It indicated that the bioactive compounds in the extracts may not interact significantly with melanin biosynthesis pathways. Additionally, the anti-inflammatory potential of *E. nitida* was investigated through NO inhibition in LPS-activated RAW 264.7 macrophages. These assays target key inflammatory pathways, with NO inhibition indicating modulation of pro-inflammatory mediators without causing cytotoxicity. This highlights the plant's selectivity in biological activity and supports its therapeutic potential, specifically in oxidative stress and inflammation-related conditions, rather than skin-lightening or pigmentation treatments. Further study on isolation should be conducted to identify bioactive compounds supporting the activity of extracts.

5. Conclusion

This research was the first to demonstrate the antioxidant, tyrosinase inhibitory, and anti-inflammatory activities of the leaf of *Eurya nitida* Korth. The EtOAc extract displayed potent antioxidant activity using ABTS and DPPH radical scavenging with IC_{50} values of 7.63 ± 0.04 and 11.10 ± 0.18 µg/mL, respectively, with high TPC and TFC values. It also displayed a good RP value. The strongest anti-inflammatory effect was observed with the EtOAc extract, without cytotoxicity, which significantly inhibited the LPS-induced production of NO (IC_{50}: 25.62 ± 1.38 µg/mL). Thereby, this extract has shown potential for treating diseases related to oxidative stress and inflammation.

Acknowledgments: *The authors gratefully acknowledge the facilities support provided by the Faculty of Pharmacy, Ton Duc Thang University, Vietnam.*

References

1. Nguyen B. H., Le T. H., Nguyen H. N. (2022), The diversity of Theaceae D. Don family in Pu Hoat Nature Reserve, Nghe An Province, *Vinh University Journal of Science*, 51(1A), 30-38.

2. Fu L., Xu B. T., Xu X. R., Qin X. S., Gan R. Y., Li H. B. (2010), Antioxidant capacities and total phenolic contents of 56 wild fruits from South China, *Molecules*, 15(12), 8602-8617.

3. Ton N. L. H., Vu H. K. L., Thach U. D., Tran T. T. T., Le T. N. T., Do D. T., Le T. K. C., Nguyen M. H. (2022), Microscopic characteristics, antioxidant activities, and chemical compositions of *Ochna integerrima* leaves and stems, *Journal of Medicinal Materials*, 27(4), 227-234.

4. Karthika K., Paulsamy S. (2015), TLC and HPTLC fingerprints of various secondary metabolites in the stem of the traditional medicinal climber, *Solena amplexicaulis*, *Indian Journal of Pharmaceutical Sciences*, 77(1), 111-116.

5. Nguyen T. H. N., Phan T. L., Le. P. T. A., Nguyen P. K., Nguyen H. A., Ton N. L. H., Nguyen B. A., Le T. K. C., Nguyen M. H. (2023), Antioxidant and anti-inflammatory potential from *Ochna integerrima* stems, *Journal of Medicinal Materials*, 28(3), 185-190.

6. Chang C. C., Yang M. H., Wen H. M., Chern J. C. (2002), Estimation of total flavonoid content in propolis by two complementary colorimetric methods, *Journal of Food and Drug Analysis*, 10(3), 3, 178-182.

7. Baliyan S., Mukherjee R., Priyadarshini A., Vibhuti A., Gupta A., Pandey R. P., Chang C. M. (2022), Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*, *Molecules*, 27(4), 1326.

8. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999), Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Biology and Medicine*, 26(9-10), 1231-1237.

9. Benzie I. F. F., Strain J. J. (1996), The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay, *Analytical Biochemistry*, 239(1), 70-76.

10. Oyaizu, M. (1986), Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine, *The Japanese Journal of Nutrition and Dietetics*, 44(6), 307-315.

11. Tsao Y. T., Hsueh Y. J., Chen H. C., Cheng C. M. (2024), Protocol for assessing total antioxidant capacity in minimal volumes of varying clinical human samples, *STAR Protocols*, 5(1), 102822.

12. Chen X., Haniu A., Kashiwagi T., Watanabe H., Watanabe T., Okamoto Y., Suzuki M., Kim C. S. (2017), The evaluation of the synergistic effect of 3-(2,4-dihydroxyphenyl)propionic acid and l-ascorbic acid on tyrosinase inhibition, *Zeitschrift fürNaturforschung. C, Journal of Biosciences*, 72(3-4), 119-121.

13. Adebayo S. A., Dzoyem J. P., Shai L. J., Eloff J. N. (2015), The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African, *BMC Complementary & Alternative Medicine*, 15(1), 1-10.

14. Kumar P., Nagarajan A., Uchil P. D. (2018), Analysis of cell viability by the MTT assay, *Cold Spring Harbor Protocols*, 2018(6), 10, 469-471.