PHYTOCHEMICAL INVESTIGATION, ANTIMICROBIAL ACTIVITY, AND CYTOTOXIC POTENTIAL OF EUPATORIUM FORTUNEI TURCZ. CULTIVATED IN TAM DAO, VIETNAM

TA VAN ĐIEP[1], TRAN HOANG KHANH HUYEN[1], PHAM MANH HA[1], CAI THI LAN HUONG[1], NGUYEN THI TUYET[1], TRAN ANH NGOC[2], TRAN DUC DAI[1*]

[1]Tan Trao University, Tuyen Quang Province, Vietnam

[2]Tam Dao Medicinal Plant Research Center, National Institute of Medicinal Materials, Phu Tho Province Vietnam

Email address: TDDai@tqu.edu.vn

**Corresponding author: Tran Duc Dai*

http://doi.org/10.51453/3093-3706/2026/1488

ARTICLE INFO

Received: **16/02/2026**
Revised: **17/03/2026**
Published: **28/4/2026**

KEYWORDS

Eupatorium fortunei;
Alkaloid;
Coumarin;
Flavonoid;
Terpenoid; Proanthocyanidin.

ABSTRACT

Eupatorium fortunei Turcz. (Asteraceae) is a medicinal plant widely used in traditional medicine and reported to possess various biological activities. However, information regarding the phytochemical composition and biological properties of *E. fortunei* cultivated at the Tam Dao Medicinal Plant Research Center remains limited. This study aimed to investigate the phytochemical constituents, antimicrobial activity, and cytotoxic potential of solvent fractions obtained from the aerial parts of *E. fortunei*. Preliminary phytochemical screening and thin-layer chromatography (TLC) analyses were performed to characterize major secondary metabolites. Antimicrobial activity was evaluated against eight standard microbial strains using the broth microdilution method, while cytotoxic activity against human cancer cell lines (HepG2, MCF-7, and A549) was assessed using the MTT assay. Phytochemical screening revealed the presence of alkaloids, coumarins, flavonoids, terpenoids, and proanthocyanidins. TLC analysis demonstrated a diverse chemical profile, with at least 10 constituents detected in the n-hexane fraction (EFH) and 16 constituents in the ethyl acetate fraction (EFE). Among the tested extracts, EFH exhibited the strongest antimicrobial activity against *Candida albicans* with a minimum inhibitory concentration (MIC) of 100 µg/mL, whereas the remaining antimicrobial effects were weak (MIC ≥ 200 µg/mL). Cytotoxicity assays showed selective activity of EFH against MCF-7 breast cancer cells (IC_{50} = 31.45 ± 1.18 µg/mL), while EFE displayed the most potent activity against A549 lung cancer cells (IC_{50} = 27.36 ± 1.05 µg/mL) and moderate activity against HepG2 cells (IC_{50} = 87.39 ± 2.51 µg/mL). These findings

demonstrate that *E. fortunei* cultivated in Tam Dao is a rich source of bioactive secondary metabolites and possesses promising antifungal and selective anticancer activities. The ethyl acetate fraction, in particular, warrants further bioassay-guided fractionation to identify compounds responsible for its cytotoxic effects against lung cancer cells.

1. INTRODUCTION

1.1. Botanical Taxon and Ethnomedicinal Background

Eupatorium fortunei Turcz. is a perennial herbaceous species belonging to the family Asteraceae and is widely distributed throughout East Asia. In Vietnam, the plant is commonly known by several vernacular names, including Huong thao, Trach lan, Lan thao, and Boi lan [1] [2].

Morphologically, *E. fortunei* is an erect perennial herb that typically grows to a height of approximately 50–100 cm. The stems are branched, smooth, pale purple in color, and possess distinct longitudinal ridges. Leaves are arranged oppositely, narrowly lanceolate in shape, and generally measure 7–11 cm in length and 1.7–2.5 cm in width, with serrated margins and purplish veins. The inflorescences consist of pale-purple capitula arranged in dense cymose clusters on pubescent peduncles located at branch terminals or leaf axils. The fruits are pentagonal blackish achenes [2].

The species is distributed in temperate and subtropical regions of China, India, Japan, Laos, and the Russian Far East . In Vietnam, *E. fortunei* occurs naturally and is cultivated on a limited scale in several northern provinces, including Lao Cai, Cao Bang, Lang Son, Phu Tho, and Thai Nguyen [1].

1.2. Traditional Uses and Pharmacological Evidence

According to traditional Chinese medicine, *E. fortunei* possesses a pungent and bitter taste and is considered neutral in nature. The plant has traditionally been used to promote blood circulation, relieve blood stasis, enhance diuresis, and reduce edema [2]. In Vietnamese traditional medicine, preparations derived from the aerial parts have been employed in the treatment of dysmenorrhea, menstrual disorders, fever, digestive disturbances, skin infections, boils, ulcers, and pruritus [1] [3].

Apart from medicinal applications, *E. fortunei* has also been utilized as a fragrant herb, a hair-cleansing agent, an insect repellent, a post-harvest preservative, and a source of essential oil for cosmetic and flavoring purposes [2].

Recent phytochemical and pharmacological investigations have revealed that *Eupatorium fortunei* Turcz. is a rich source of structurally diverse secondary metabolites, including terpenoids, flavonoids, phenylpropanoids, pyrrolizidine alkaloids, benzofuran derivatives, and thymol-related compounds. These phytoconstituents are considered to underpin the broad spectrum of biological activities reported for this species, including anti-inflammatory, antiviral, antifungal, antidiabetic, and anticancer effects [4]. In particular, several compounds isolated from *E. fortunei* have demonstrated notable anti-inflammatory properties through the inhibition of nitric oxide (NO) production and the modulation of key inflammatory signaling pathways, such as NF-κB and p38 MAPK [5], [6].

Although recent reviews have also cited antioxidant, antimicrobial, and antidiabetic activities associated with *E. fortunei*, the primary experimental evidence supporting these pharmacological effects remains relatively limited and, in some cases, insufficiently characterized. Therefore, the currently available data should be interpreted with caution, and further well-designed mechanistic and in vivo studies are required to substantiate these therapeutic claims and clarify their underlying molecular mechanisms [4].

1.3. Rationale and Study Objectives

The accumulation of secondary metabolites in medicinal plants is strongly influenced by both genetic and environmental factors. Geographic location, soil composition, altitude, temperature, precipitation, solar radiation, and cultivation practices can significantly affect phytochemical composition and consequently alter biological activity. Therefore, plants of the same species cultivated under different environmental conditions may exhibit substantial variation in their chemical profiles and pharmacological properties [7], [8], [9].

To date, no published studies have comprehensively evaluated the phytochemical characteristics and biological activities of *E. fortunei* cultivated at the Tam Dao Medicinal Plant Research Center, National Institute of Medicinal Materials, Vietnam. Located in a mountainous region characterized by unique climatic and edaphic conditions, Tam Dao represents an important center for medicinal plant conservation and cultivation. Characterization of *E. fortunei* grown under these conditions may contribute to quality control, phytochemical standardization, and future pharmaceutical development.

Therefore, the present study was conducted with the following objectives:

(1) To perform qualitative phytochemical screening and establish thin-layer chromatography (TLC) profiles of the n-hexane and ethyl acetate fractions obtained from the aerial parts of *E. fortunei*.

(2) To determine the minimum inhibitory concentration (MIC) values of these fractions against selected Gram-positive bacteria, Gram-negative bacteria, and fungal strains using the broth microdilution method.

(3) To evaluate the cytotoxic and antiproliferative activities of these fractions against human hepatocellular carcinoma (HepG2), breast adenocarcinoma (MCF-7), and non-small cell lung cancer (A549) cell lines using the MTT assay.

2. RELATED WORKS

2.1. Phytochemical Studies of *Eupatorium fortunei*

Phytochemical investigations of *Eupatorium fortunei* have revealed a diverse array of both volatile and non-volatile constituents. Analyses of the essential oil have identified *p*-cymene, thymol, β-caryophyllene, thymol methyl ether, and several oxygenated monoterpenes as major components, which contribute to the characteristic aroma of the plant and may be associated with some of its traditional medicinal applications [4].

More recent phytochemical studies have demonstrated that *E. fortunei* is a rich source of structurally diverse secondary metabolites, including terpenoids, sesquiterpenes, thymol derivatives, flavonoids, phenylpropanoids, coumarins, benzofuran derivatives, and pyrrolizidine alkaloids. Several phenolic constituents and flavonoids have also been identified from this species, and these

compounds are considered important contributors to its reported pharmacological activities, particularly its antioxidant and anti-inflammatory properties [5], [6], [4].

Despite increasing interest in the species, most phytochemical studies have been conducted using plant materials collected in China, Japan, or Korea. Information regarding the phytochemical characteristics of *E. fortunei* cultivated under the environmental conditions of Tam Dao, Vietnam, remains scarce.

2.2. Antimicrobial and Antifungal Activities

Members of the family Asteraceae are widely recognized as rich sources of bioactive secondary metabolites, including terpenoids, flavonoids, phenolic compounds, and essential oil constituents, many of which have been reported to possess antimicrobial properties. These compounds may inhibit microbial growth through multiple mechanisms, such as disruption of cell membrane integrity, interference with metabolic processes, and modulation of enzyme activity [9], [4].

Phytochemical and pharmacological studies of *Eupatorium fortunei* have suggested that extracts and essential oil components from this species may exhibit antimicrobial potential. The volatile oil of *E. fortunei*, which is rich in thymol derivatives, *p*-cymene, and other terpenoid constituents, has been proposed as a possible source of antimicrobial activity. However, the currently available evidence supporting antibacterial activity against specific pathogenic microorganisms remains limited, and comprehensive evaluations using standardized antimicrobial assays are still lacking. Likewise, reports concerning antifungal activity are scarce, and the available data are insufficient to draw definitive conclusions regarding the efficacy of *E. fortunei* against clinically relevant fungal pathogens. Therefore, further investigations are required to clarify its antimicrobial spectrum, active constituents, and underlying mechanisms of action [4].

Furthermore, relatively few studies have systematically compared the antimicrobial activities of fractions obtained using solvents of different polarities. Comparative evaluation of *n*-hexane and ethyl acetate fractions may therefore provide useful information regarding the distribution of bioactive compounds within the plant.

2.3. Cytotoxic and Antiproliferative Activities

The genus *Eupatorium* has attracted considerable interest as a promising source of anticancer agents owing to its rich diversity of bioactive terpenoids and related secondary metabolites. Numerous compounds isolated from *Eupatorium* species, particularly sesquiterpenes and thymol derivatives, have been reported to exhibit cytotoxic effects against various human cancer cell lines through mechanisms involving apoptosis induction, inhibition of cell proliferation, and modulation of cellular signaling pathways [4].

For *Eupatorium fortunei* specifically, several phytochemicals isolated from the aerial parts have demonstrated moderate to significant cytotoxic activity against human tumor cell lines in vitro. Notably, a number of thymol derivatives and other terpenoid constituents exhibited growth-inhibitory effects against lung, breast, liver, and cervical cancer cell models [5], [6], [4]. Although the precise molecular mechanisms remain incompletely characterized, available evidence suggests that these effects may be associated with the regulation of oxidative stress responses and apoptosis-related signaling pathways. Further mechanistic and in vivo studies are required to validate the

therapeutic potential of these compounds and clarify their modes of action [4].

Although these findings suggest considerable pharmacological potential, information regarding the cytotoxic and antiproliferative activities of *E. fortunei* cultivated in Vietnam remains extremely limited. Therefore, further studies are required to evaluate the biological activities of locally cultivated material and to determine whether environmental conditions influence its bioactive properties.

2. Materials and Methods

2.1. Plant Materials

Figure 2.1. *Eupatorium fortunei* Turcz. cultivated in the Medicinal Plant Conservation Garden, Tam Dao Medicinal Plant Research Center, September 2025.

The plant material used in this study consisted of the aerial parts of *Eupatorium fortunei* Turcz., including stems, leaves, and flowers.

Collection site: Medicinal Plant Conservation Garden, Tam Dao Medicinal Plant Research Center, National Institute of Medicinal Materials, Vietnam.

Collection period: September 2025.

The plant specimen was taxonomically identified as *Eupatorium fortunei* Turcz. by Dr. Do Cong Ba, Faculty of Medicine and Pharmacy, Tan Trao University. A voucher specimen (EF-2025) was deposited and preserved at the Pharmaceutical Practice Center, Tan Trao University, for future reference and verification.

2.2. Materials, Reagents, Equipment, and Experimental Methods

2.2.1. Instruments, Reagents, and Equipment for Plant Extraction

Instruments and Equipment:

The instruments and equipment employed in this study included a drying oven, hot-air dryer, electric heating plate, water bath, rotary vacuum evaporator, precision balance, analytical balance, optical microscope, microtome blades (razor blades), a 20-L ultrasonic bath, essential oil

distillation apparatus, reagent sprayer, ultraviolet (UV) lamp for thin-layer chromatography (TLC) visualization at wavelengths of 254 and 365 nm, glass containers, beakers, Erlenmeyer flasks, separatory funnels, test tubes, pipettes, wooden test-tube holders, glass stirring rods, enamel trays, porcelain mortars, filter papers, and silica gel TLC plates.

Chemicals and Reagents:

The chemicals and reagents used in this study included n-hexane, 96% ethanol, methanol, chloroform, ethyl acetate, formic acid, 70% ethanol, Mayer's reagent, 5% ferric chloride ($FeCl_3$) solution, 10% sodium hydroxide (NaOH) solution, hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), 1% gelatin solution, Bouchardat reagent (I_2/KI), sodium hypochlorite solution (Javel solution), 5% acetic acid solution, carmine red stain, and methylene blue stain.

All chemicals and solvents used throughout the study were of analytical grade.

2.2.2. Extraction and Preparation of Plant Extracts

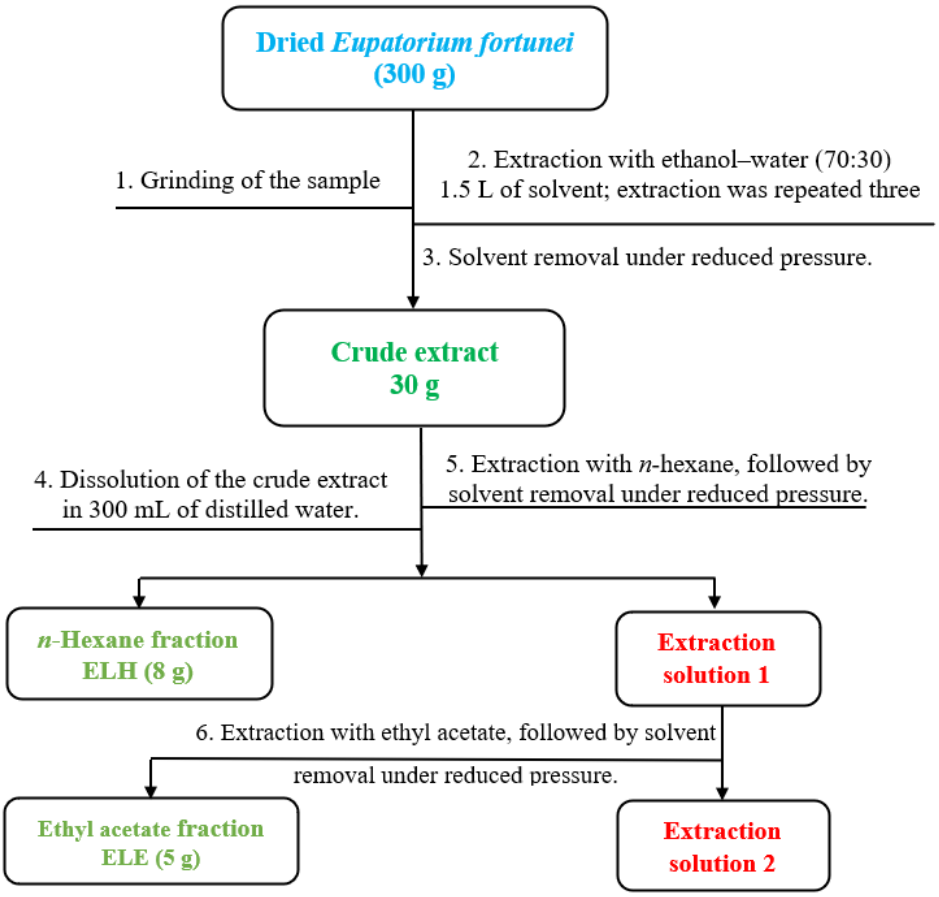


Figure 2.2. Schematic representation of the extraction and fractionation procedure for the aerial parts of *Eupatorium fortunei* Turcz.

All solvents used during the extraction process were of analytical-grade purity. The extraction and fractionation procedures for the aerial parts of *Eupatorium fortunei* Turcz. were conducted according to the workflow illustrated in **Figure 2.2**.

2.2.3. Preliminary Phytochemical Screening

In this study, the chemical constituents present in *Eupatorium fortunei* were preliminarily screened following the standard procedures described in the Vietnamese Pharmacopoeia V [10].

After obtaining the crude extracts and fractions, the presence of phytochemical groups was identified through characteristic colorimetric and precipitation reactions using specific reagents. All experiments were performed independently in triplicate to ensure data reliability and reproducibility.

Preparation of test solution: Three grams of each extract were dissolved in 70% ethanol in a 250 mL glass beaker. After complete dissolution, the mixture was allowed to stand for sedimentation and then filtered through filter paper. The filtrate was collected in a 250 mL Erlenmeyer flask and used as the test solution for subsequent qualitative assays.

Alkaloids detection: Two milliliters of the test solution were placed in a test tube, followed by the addition of three drops of Bouchardat's reagent (I_2/KI). The formation of a brownish-red to orange precipitate indicated a positive result.

Coumarins detection: Two milliliters of the test solution were treated with a few drops of 10% NaOH solution, followed by acidification with HCl. The appearance of a white precipitate was considered a positive indication.

Cardiac glycosides detection: Two milliliters of the test solution were mixed with a few drops of $FeCl_3$ solution and shaken gently. The test tube was inclined at approximately 45°, and concentrated H_2SO_4 was carefully added along the wall to form a distinct two-layer system. The appearance of a brownish-red ring at the interface of the two layers indicated a positive reaction.

Flavonoids detection: Two milliliters of the test solution were mixed with three drops of 5% $FeCl_3$ solution. The development of a dark green, blue-black, or bluish-violet coloration confirmed the presence of flavonoids.

Proanthocyanidins detection: Two milliliters of the test solution were mixed with 5 mL of 10% HCl and heated in a water bath for 10 min. The formation of a red or pink-red coloration indicated a positive result.

Anthocyanins detection: Two milliliters of the test solution were acidified with a few drops of 10% HCl, resulting in an enhanced red coloration. Subsequent addition of NaOH solution leading to a blue color change confirmed the presence of anthocyanins.

Tannins detection: Two milliliters of the test solution were mixed with 1 mL of 1% gelatin solution and gently stirred. The formation of a white precipitate indicated a positive reaction.

Saponins detection: Five milliliters of the test solution were vigorously shaken for 30 seconds and then allowed to stand. Persistent foam formation lasting for at least 15 minutes was considered indicative of saponins.

Terpenoids detection: Two milliliters of the test solution were mixed with 2 mL of chloroform, followed by the slow addition of concentrated H_2SO_4. The appearance of a colored ring or green layer at the interface of the two phases indicated a positive reaction.

2.2.4. Thin-Layer Chromatography (TLC) Analysis of Phytochemical Constituents

Thin-layer chromatography (TLC) is a widely used analytical technique in phytochemical studies for the preliminary identification and profiling of bioactive compounds in plant extracts. The principle of TLC is based on the differential partitioning of compounds between a stationary phase (a thin layer of adsorbent, typically silica gel coated on aluminum, glass, or plastic plates) and a mobile phase (an appropriate solvent system). As the mobile phase migrates through capillary

action, compounds are separated according to their relative affinity for the stationary and mobile phases, resulting in distinct chromatographic spots [11].

TLC procedure: Silica gel TLC plates were activated by drying at an appropriate temperature to remove moisture prior to use. The plant extracts were dissolved in suitable solvents and spotted onto the plates using capillary tubes at a distance of approximately 1 cm from the lower edge.

The developing chamber was saturated with the selected mobile phase system. After equilibrium was achieved, the TLC plate was placed vertically in the chamber, ensuring that the sample spots were positioned above the solvent level. Development was carried out in a closed chamber until the solvent front reached the designated height.

After development, the plates were removed, and the solvent front was immediately marked. The plates were air-dried at room temperature. Chromatographic spots were visualized under ultraviolet (UV) light at 254 nm and 365 nm to detect UV-absorbing or fluorescent compounds.

To enhance visualization of organic constituents, the plates were further sprayed with H_2SO_4 reagent and heated until characteristic colored spots appeared. The retention factor (Rf) values and color characteristics of each spot were recorded and used for preliminary identification of phytochemical groups present in the samples [11].

Data processing: The retention factor (Rf) was calculated using the following equation:

$$R_f = a/b$$

Where:

+ R_f: retention factor of the analyte;

+ a: distance travelled by the analyte (from the application point to the center of the spot);

+ b: distance travelled by the solvent front (from the application point to the solvent front).

TLC results were evaluated based on the number of spots, color characteristics, fluorescence behavior under UV light, and the Rf values of individual chromatographic spots.

2.2.5. Evaluation of antimicrobial activity

The antimicrobial activity of extracts from *Eupatorium fortunei* Turcz. was evaluated using the broth microdilution method in 96-well microtiter plates to determine the Minimum Inhibitory Concentration (MIC). The assays were performed at the Experimental Biology Laboratory, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, following the methods described by Vanden Berghe and Vlietinck (1991) and McKane and Kandel (1996) [12], [13].

Test microorganisms: Antimicrobial activity was assessed against eight standard microbial strains, including:

+ Gram-negative bacteria: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

+ Gram-positive bacteria: *Bacillus subtilis* (ATCC 11774) and *Staphylococcus aureus* subsp. *aureus* (ATCC 11632).

+ Filamentous fungi: *Aspergillus niger* (439) and *Fusarium oxysporum* (M42).

+ Yeasts: Candida albicans (ATCC 7754) and Saccharomyces cerevisiae (SH20).

Experimental controls: Positive controls included streptomycin for Gram-positive bacteria,

tetracycline for Gram-negative bacteria, and nystatin or amphotericin B for yeasts and filamentous fungi. All reference compounds were dissolved in dimethyl sulfoxide (DMSO) at appropriate concentrations.

Negative controls consisted of the corresponding solvent used to dissolve the tested samples without any active compound or reference antibiotic.

Assay procedure: Microbial strains were activated on appropriate culture media prior to testing. Inocula were standardized in sterile physiological saline to match a 0.5 McFarland standard.

Extract samples were dissolved in DMSO and serially diluted in decreasing concentrations across 96-well microplates. After inoculation with microbial suspensions, plates were incubated at 37°C for 24 h for bacterial strains and at 30°C for 48 h for fungal strains.

Data interpretation: Antimicrobial activity was expressed as MIC (Minimum Inhibitory Concentration), defined as the lowest concentration of the tested sample that completely or nearly completely inhibits visible microbial growth after incubation [12], [13].

All experiments were performed independently in triplicate. Results were reported as MIC values (µg/mL or µg/well) for each sample against each tested microorganism.

2.2.6. Evaluation of cytotoxicity and antiproliferative activity using the MTT assay

Cytotoxic and antiproliferative activities of the investigated samples were evaluated using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], a widely used in vitro screening method for anticancer potential, recommended by the National Cancer Institute (NCI, USA) [14].

Principle: The MTT assay is based on the reduction of the yellow tetrazolium salt MTT into insoluble purple formazan crystals by mitochondrial dehydrogenase enzymes dependent on NAD(P)H in viable cells. The amount of formazan produced is directly proportional to the number of metabolically active cells. After dissolution of formazan crystals in dimethyl sulfoxide (DMSO), absorbance is measured at 540 nm with a reference wavelength of 720 nm. A decrease in optical density relative to the negative control reflects cytotoxic or antiproliferative effects of the tested samples [14].

Cell line: The human hepatocellular carcinoma cell line Hep-G2 was used to evaluate cytotoxic activity. The cell line was obtained from international cell banks, including ATCC (USA) and CLS (Germany), and is currently maintained at the Experimental Biology Laboratory, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

Cell culture conditions: Hep-G2 cells were cultured under sterile conditions at 37°C in a humidified atmosphere containing 5% CO_2. Culture media included DMEM, EMEM (Sigma-Aldrich, USA), or RPMI-1640 (Thermo Fisher Scientific, Germany), supplemented with 5–10% fetal bovine serum (FBS), 2 mM L-glutamine, and a penicillin–streptomycin antibiotic mixture.

Experimental procedure: Cells were seeded in 96-well plates at a density of 1.5×10^5 cells/well and allowed to stabilize prior to treatment.

Extracts were tested over a concentration range of 6.25–100 µg/mL. For pure compounds, the tested range was 1–50 µM. Each concentration was assayed in triplicate wells.

Positive controls included ellipticine or paclitaxel (Taxol), dissolved in DMSO and used according to standard laboratory protocols. Negative controls consisted of cells cultured under

identical conditions without test compounds.

After incubation, MTT reagent was added to each well and further incubated to allow formation of formazan crystals. The culture medium was then removed, and crystals were fully dissolved in DMSO. Absorbance was measured using an Infinite F50 microplate reader (Tecan, Männedorf, Switzerland) at 540 nm with a 720 nm reference wavelength (Mosmann, 1983).

Data analysis:

Cell inhibition (%) = [1 − (OD[sample] /OD[negative control])] × 100%

Where:

+ OD[sample]: optical density of wells containing test samples;

+ OD[negative control]: optical density of control wells without test compounds.

Results were expressed as mean ± standard deviation (Mean ± SD) from three independent experiments.

$$\sigma = \sqrt{\left(\Sigma\left(x_i - \bar{x}\right)^2\right)/(n-1)}$$

Samples exhibiting cytotoxic activity with an inhibition rate of ≥ 50% at one or more investigated concentrations will be further evaluated to determine their IC_{50}. The IC_{50} value is defined as the concentration of the test sample required to inhibit 50% of cell viability compared to the negative control.

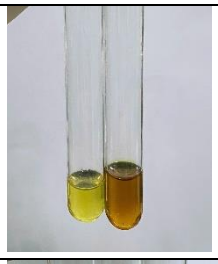
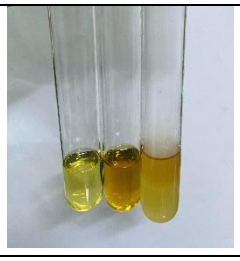
IC_{50} values are determined by non-linear regression analysis using TableCurve 2D software (Jandel Scientific, San Rafael, CA, USA) [15].

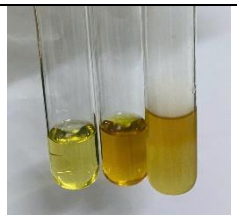
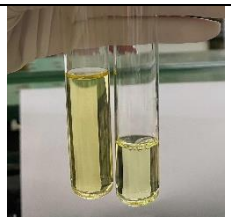
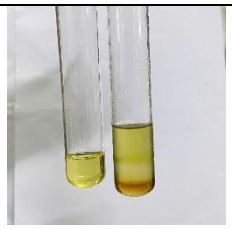
3. Results and Discussion

3.1. Preliminary phytochemical screening results

The preliminary phytochemical screening of chemical constituents in the extracts of *Eupatorium fortunei* Turcz. is presented in Table 3.1. The qualitative tests were based on the observation of characteristic reactions specific to each class of compounds, including precipitate formation, color changes, or the appearance of specific colored rings.

Table 3.1. Preliminary phytochemical screening of chemical constituents in *Eupatorium fortunei* extracts.

No	Substance group	Qualitative reaction	Result	Illustrative image
1	Alkaloid	Reaction with bouchardat reagent	+++	
2	Coumarin	Reaction with 10% NaOH and acidification with HCl	+++	

3	Glycosid tim	Reaction with Keller-Kiliani	-	
4	Flavonoid	Reaction with dung dịch $FeCl_3$	+++	
5	Proanthocyanidin	React with 10% HCl and heat in a water bath.	+	
6	Anthocyanin	Reaction with 10% HCl and NaOH	-/+	
7	Tanin	Reaction with gelatin 1%	-	
8	Saponin	Foaming reaction	-	
9	Terpenoid	Reaction with chloroform and concentrated H_2SO_4	+++	

Symbols: (-): negative; (+): weakly positive; (++): clearly positive; (+++): very clearly positive; (-/+): unclear reaction.

3.1. Preliminary Phytochemical Screening Results

The preliminary phytochemical screening revealed the presence of several important classes

of secondary metabolites in the *Eupatorium fortunei* extract, including alkaloids, coumarins, flavonoids, proanthocyanidins, and terpenoids. Among these, flavonoids, alkaloids, and terpenoids exhibited strongly positive reactions (+++), suggesting that these compounds may constitute major components of the extract.

The occurrence of flavonoids in *E. fortunei* extract is consistent with previous studies on the genus *Eupatorium*, in which flavonoids have been recognized as one of the predominant phytochemical groups responsible for a wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects [16]. Flavonoids are capable of scavenging free radicals, chelating transition metal ions, and modulating signaling pathways involved in inflammatory responses [17].

The positive detection of terpenoids is also in agreement with the characteristic phytochemical profile of species belonging to the Asteraceae family. Numerous studies have demonstrated that terpenoids, particularly sesquiterpenoids isolated from *Eupatorium* species, exhibit diverse biological activities, including antimicrobial, anti-inflammatory, and cytotoxic effects through various mechanisms of action [18]. Therefore, terpenoids may contribute substantially to the biological activities observed in *E. fortunei* extracts during subsequent bioassays.

A strongly positive reaction (+++) was also observed for alkaloids. Alkaloids constitute a diverse class of nitrogen-containing natural products with significant pharmacological potential. Many naturally occurring alkaloids have been reported to exhibit antimicrobial activity, regulate enzymatic processes, and interfere with cellular proliferation pathways [19].

In addition, the extract tested positive for coumarins and proanthocyanidins. Coumarins are phenolic compounds capable of absorbing ultraviolet radiation and are commonly found in numerous members of the Asteraceae family. Previous reports have demonstrated their antioxidant, anti-inflammatory, and antimicrobial properties [20]. Proanthocyanidins, which are polymeric flavonoids, are well known for their free-radical scavenging capacity and their role in protecting cells against oxidative damage [18].

In contrast, the Keller–Kiliani test for cardiac glycosides, the frothing test for saponins, and the gelatin precipitation test for tannins all yielded negative results. These findings suggest that these classes of compounds were either absent from the extract or present at concentrations below the detection limit of the qualitative assays employed. The anthocyanin test produced an inconclusive result (-/+), which may be attributed to the low abundance of anthocyanin pigments or to the influence of extraction conditions and reaction medium pH on pigment stability.

Overall, the preliminary phytochemical screening demonstrated that *E. fortunei* contains a variety of biologically relevant secondary metabolites, particularly flavonoids, terpenoids, alkaloids, coumarins, and proanthocyanidins. The presence of these phytochemical groups provides a scientific basis for further investigations into the antimicrobial, cytotoxic, and other biological activities of this medicinal plant.

3.2. Identification of Phytochemical Constituents in the *n*-Hexane and Ethyl Acetate Extracts by Thin-Layer Chromatography (TLC)

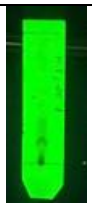
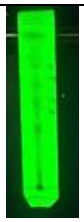
3.2.1. Phytochemical Profiling of the *n*-Hexane Fraction by Thin-Layer Chromatography

(TLC)

The phytochemical constituents of the n-hexane fraction obtained from *Eupatorium fortunei* were investigated using thin-layer chromatography (TLC) on silica gel plates as the stationary phase. Chromatographic separation was evaluated using a series of *n*-hexane:ethyl acetate (H:E) solvent systems with progressively increasing polarity, including volume ratios of 20:1, 15:1, 9:1, 7:1, 5:1, 3:1, 2:1, and 1:1.

Experimental results indicated that the solvent systems H:E = 20:1 and H:E = 5:1 provided the most effective separation, producing well-resolved and distinct chromatographic spots. Detailed chromatographic characteristics of the *n*-hexane fraction under these solvent conditions are presented in **Table 3.2**.

Table 3.2. TLC profiles of the *n*-hexane fraction of *Eupatorium fortunei*.

Solvent system	**H:E 20:1**	**H:E 5:1**
Wavelength λ = 365 nm		
Wavelength λ = 254 nm		

3.2.1. Phytochemical Profiling of the *n*-Hexane Fraction by Thin-Layer Chromatography (TLC)

When TLC was developed using the low-polarity solvent system *n*-hexane:ethyl acetate (H:E = 20:1), observation under UV light at λ = 365 nm revealed at least seven fluorescent spots distributed predominantly in the upper and middle regions of the chromatographic plate, exhibiting relatively uniform fluorescence intensity. This observation indicates that the n-hexane fraction contains at least seven lipophilic compounds present in comparable amounts, which were effectively eluted by this solvent system.

Increasing the polarity of the mobile phase to H:E = 5:1 enhanced the elution strength, facilitating the desorption and migration of moderately polar compounds that remained strongly retained near the origin in the H:E = 20:1 system. Under UV illumination at 365 nm, a broad chromatographic band was resolved into at least ten distinct fluorescent spots displaying characteristic bright red fluorescence. Among these, the spots with Rf values of approximately 0.5, 0.8, and 0.9 exhibited notably larger spot areas, stronger coloration, and greater definition than the others. Such optical characteristics are commonly associated with compounds possessing extensive conjugated π-electron systems or porphyrin ring structures, including natural pigments such as chlorophylls and their pheophytin derivatives [21], [22]. The intense coloration and large spot sizes observed at these Rf values suggest that these compounds constitute major constituents of the extract fraction.

Furthermore, examination under UV light at λ = 254 nm revealed pronounced fluorescence quenching in both solvent systems, resulting in well-defined dark spots against the green fluorescent background of the silica gel plate. This phenomenon provides evidence for the presence of compounds containing aromatic ring systems or conjugated double-bond structures capable of strongly absorbing UV radiation at λ = 254 nm. These constituents are likely to include free coumarins (e.g., coumarin and *o*-coumaric acid derivatives) and low-polarity terpenoids, which are recognized as characteristic secondary metabolites of *Eupatorium fortunei* [23], [24].

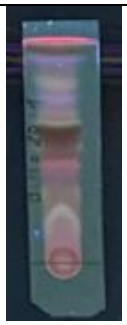
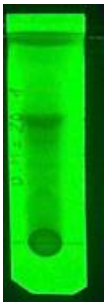
Overall, the *n*-hexane fraction of *Eupatorium fortunei* contains a chemically diverse mixture of low-to medium-polarity constituents. At least ten compounds were clearly resolved using the H:E = 5:1 solvent system, with natural chlorophyll-related pigments and compounds possessing aromatic or conjugated structures (such as coumarins and terpenoids) appearing to be the predominant components.

3.2.2. Identification of Chemical Constituents in the Ethyl Acetate Fraction by Thin-Layer Chromatography (TLC)

The chemical composition of the ethyl acetate fraction obtained from *Eupatorium fortunei* was investigated by thin-layer chromatography (TLC) using silica gel as the stationary phase. The mobile phase consisted of dichloromethane:methanol (D:M) mixtures evaluated over a range of solvent ratios with progressively increasing polarity, including 30:1, 20:1, 15:1, 9:1, 7:1, 5:1, 3:1, 2:1, and 1:1 (v/v).

Among the solvent systems tested, D:M = 30:1 and D:M = 20:1 provided the most effective chromatographic separation, yielding well-resolved spots with superior resolution and spot definition. Detailed TLC characteristics of the ethyl acetate fraction are presented in Table 3.3.

Table 3.3. TLC characteristics of the ethyl acetate extract fraction of *Eupatorium fortunei*.

Solvent system	**D: M = 30: 1**	**D: M = 20: 1**
Wavelength λ = 365 nm		
Wavelength λ = 254 nm		

3.2.2. Identification of Chemical Constituents in the Ethyl Acetate Fraction by Thin-Layer Chromatography (TLC)

Email: sjttu@tqu.edu.vn

When TLC was developed using the dichloromethane:methanol solvent system (D:M = 30:1), observation under UV light at λ = 365 nm revealed the preliminary separation of at least ten fluorescent spots with relatively comparable intensities. This result indicates that this moderately low-polarity mobile phase was capable of effectively separating constituents with low-to-moderate polarity present in the ethyl acetate fraction.

Upon increasing the methanol content to obtain a more polar solvent system (D:M = 20:1), the desorption and elution capacity of the mobile phase toward moderately polar compounds increased substantially. Under UV illumination at λ = 365 nm, a greater number of chromatographic spots became visible, with at least sixteen distinct fluorescent spots clearly resolved. Notably, the spots located in the central region of the TLC plate, corresponding to Rf values of approximately 0.4, 0.5, and 0.6, exhibited larger spot sizes, higher concentration density, and markedly stronger fluorescence intensity than the remaining spots. These observations suggest that compounds corresponding to these Rf values represent major constituents of the fraction.

Previous phytochemical studies have shown that ethyl acetate extracts of *Eupatorium fortunei* are typically enriched in free flavonoids, phenolic acids, and moderately polar coumarin derivatives. The occurrence of multicolored fluorescence under UV light at λ = 365 nm is widely recognized as a characteristic indicator of these highly conjugated polycyclic structures [24], [21].

In parallel, examination under UV light at λ = 254 nm demonstrated strong UV absorption by the separated constituents in both solvent systems, resulting in well-defined quenched spots (dark zones) against the fluorescent green background of the silica gel plate. This phenomenon provides additional evidence that the separated compounds possess strong UV-absorbing chromophores, such as aromatic ring systems and conjugated carbon–carbon double-bond structures [23].

Overall, the ethyl acetate fraction of *Eupatorium fortunei* contains a highly diverse array of moderately polar phytochemicals. The solvent system D:M = 20:1 exhibited the best chromatographic resolution for this fraction, enabling the clear detection of at least sixteen compounds. The dominant constituents are likely polycyclic compounds with stable conjugated structures, particularly flavonoids and phenolic compounds [24] [25], [23].

3.3. Antibacterial and Antifungal Activities

The antimicrobial activities of the *n*-hexane fraction (EFH) and ethyl acetate fraction (EFE) obtained from *Eupatorium fortunei* were evaluated against a panel of bacterial and fungal microorganisms using the minimum inhibitory concentration (MIC) assay described in Section 2.2.5. MIC values determined against Gram-negative bacteria, Gram-positive bacteria, filamentous fungi, and yeasts are summarized in Table 3.4.

Table 3.4. Minimum inhibitory concentration (MIC, µg/mL) values of extract fractions from *Eupatorium fortunei*.

No	Sample symbols	Highest concentration in the test sample (µg/ml)	Minimum inhibitory concentration (MIC: µg/ml)							
			Gram-negative bacteria		Gram-positive bacteria		Mold		Yeast	
			E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*	*A. niger*	*F. oxysporum*	*S. cerevisiae*	*C. albicans*

Email: sjttu@tqu.edu.vn

	EFH	200	> 200	> 200	200	> 200	> 200	200	> 200	100
	EFE	200	200	> 200	> 200	> 200	> 200	200	> 200	200

Based on the experimental results presented in Table 3.4, both the *n*-hexane fraction (EFH) and the ethyl acetate fraction (EFE) of *Eupatorium fortunei* exhibited weak to moderate antimicrobial activity, with MIC values generally ranging from 100 to 200 µg/mL.

***n*-Hexane fraction (EFH):** Among the tested microorganisms, EFH demonstrated the strongest inhibitory activity against the pathogenic yeast *Candida albicans* (MIC = 100 µg/mL). At the highest tested concentration (200 µg/mL), EFH also inhibited the growth of the Gram-positive bacterium *Bacillus subtilis* and the filamentous fungus *Fusarium oxysporum*. In contrast, no detectable activity was observed against the remaining microorganisms, including the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, the Gram-positive bacterium *Staphylococcus aureus*, and the yeast *Saccharomyces cerevisiae* (MIC > 200 µg/mL).

The susceptibility of *C. albicans* and *B. subtilis* to EFH may be attributed to the enrichment of relatively non-polar constituents, such as essential oils, terpenoids, and free coumarins, within this fraction [21]. These lipophilic compounds can readily interact with and disrupt microbial lipid membranes, resulting in leakage of intracellular contents and subsequent inhibition of microbial growth [25].

Ethyl acetate fraction (EFE): The antimicrobial profile of EFE differed somewhat from that of EFH. This fraction exhibited inhibitory activity only at the upper concentration limit against *Escherichia coli*, *Fusarium oxysporum*, and *Candida albicans* (MIC = 200 µg/mL). Unlike EFH, EFE showed activity against *E. coli* but was inactive against *B. subtilis*. This observation may be explained by the chemical composition of the ethyl acetate fraction, which is expected to be enriched in moderately polar constituents such as flavonoids and phenolic acids (Marston A. , 2011). These compounds exert antimicrobial effects through several mechanisms, including complex formation with cell wall proteins, enzyme inhibition, and disruption of membrane integrity [26]. Nevertheless, the highly selective outer membrane characteristic of Gram-negative bacteria such as *E. coli* may limit the penetration of bioactive compounds, thereby necessitating relatively high concentrations to achieve microbial inhibition.

Overall, the *n*-hexane fraction (EFH) exhibited greater antifungal activity against *Candida albicans* than the ethyl acetate fraction (EFE). However, the antimicrobial potency of both crude fractions remained relatively modest. Further bioassay-guided fractionation and purification are therefore required to isolate individual compounds with enhanced antimicrobial efficacy.

As shown in Table 3.4, EFH displayed the most pronounced inhibitory effect against *Candida albicans* (MIC = 100 µg/mL). At a moderate activity level (MIC = 200 µg/mL), EFE inhibited three test microorganisms (*E. coli*, *F. oxysporum*, and *C. albicans*), whereas EFH additionally exhibited activity against *B. subtilis* and *F. oxysporum*. These findings provide scientific support for the traditional use of *E. fortunei* in folk medicine and traditional medicine for the treatment of boils, skin ulcers, and infectious inflammatory conditions [1], [2].

Given the increasing prevalence of multidrug-resistant pathogens resulting from the widespread misuse of antibiotics, *E. fortunei* may represent a promising natural source of

antimicrobial agents warranting further investigation.

3.4. Cytotoxic and Antiproliferative Activities (MTT Assay)

The in vitro cytotoxic and antiproliferative activities of the *n*-hexane fraction (EFH) and ethyl acetate fraction (EFE) obtained from *Eupatorium fortunei* Turcz. were evaluated against three human cancer cell lines, namely hepatocellular carcinoma (HepG2), breast adenocarcinoma (MCF-7), and lung carcinoma (A549), using the MTT assay. The experimental procedure is described in detail in Section 2.2.6.

The effects of the extracts on cell viability, together with their corresponding half-maximal inhibitory concentration (IC_{50}) values, are summarized in Table 3.5.

Table 3.5. Cytotoxic activity of the *n*-hexane and ethyl acetate fractions of *Eupatorium fortunei* Turcz. against human cancer cell lines.

TT	Sample symbols	IC_{50} Value (µg/mL)		
		HepG2 Liver cancer	MCF-7 Breast cancer	A549 Lung cancer
	DMSO	100		
	Ellipticine	0.46 ± 0.01	0.44 ± 0.02	0.51 ± 0.04
1	EFH	> 100	31.45 ± 1.18	> 100
2	EFE	87.39 ± 2.51	> 100	27.36 ± 1.05

The IC_{50} value (µg/mL), defined as the concentration required to inhibit 50% of cell growth, is a key parameter for evaluating the anticancer potential of tested agents. According to the screening criteria established by the U.S. National Cancer Institute (NCI) for crude plant extracts, a fraction is considered to possess promising cytotoxic activity when its IC_{50} value is below 30 µg/mL [14], [27].

Positive and negative controls: The assay system demonstrated high reliability and validity, as the positive control, ellipticine, exhibited potent cytotoxic activity against all three cancer cell lines, namely HepG2, MCF-7, and A549, with remarkably low IC_{50} values of 0.46 ± 0.01, 0.44 ± 0.02, and 0.51 ± 0.04 µg/mL, respectively [28]. In contrast, the negative control (DMSO, used as the solvent vehicle) showed no significant cytotoxicity at the tested concentration (IC_{50} > 100 µg/mL), confirming that the observed effects were attributable to the extracts rather than the solvent.

***n*-Hexane fraction (EFH):** The EFH fraction exhibited moderate and selective cytotoxicity toward the human breast cancer cell line MCF-7, with an IC_{50} value of 31.45 ± 1.18 µg/mL. However, this fraction was essentially inactive or only weakly active against HepG2 and A549 cells (IC_{50} > 100 µg/mL). The enhanced sensitivity of MCF-7 cells to the relatively non-polar EFH fraction may be associated with the presence of lipophilic constituents such as essential oil components, sterols, and free triterpenoids, which have been reported to exhibit high affinity for breast cancer cell membranes or to interfere with intracellular signaling pathways involved in breast cancer progression [29], [30], [31].

Ethyl acetate fraction (EFE): The EFE fraction displayed a distinctly different cytotoxic profile and demonstrated greater anticancer potential than EFH. Notably, EFE exhibited the strongest cytotoxic effect against the human lung cancer cell line A549, with an IC_{50} value of 27.36

± 1.05 μg/mL. This value satisfies the NCI criterion for promising cytotoxic activity in crude plant extracts (IC_{50} < 30 μg/mL). In addition, EFE showed moderate inhibitory activity against HepG2 cells (IC_{50} = 87.39 ± 2.51 μg/mL) but was inactive against MCF-7 cells (IC_{50} > 100 μg/mL).

The pronounced cytotoxicity of EFE toward A549 cells may be attributed to the accumulation of moderately polar phenolic constituents, particularly flavonoids, which are known to occur abundantly in *Eupatorium fortunei*. Such compounds have been reported to induce apoptosis, arrest cell-cycle progression, and promote intracellular reactive oxygen species (ROS) accumulation in cancer cells, thereby contributing to their anticancer effects [32].

Overall, the ethyl acetate fraction (EFE) demonstrated the most promising cytotoxic activity, particularly against the human lung cancer cell line A549, achieving an IC_{50} value that meets the screening threshold for bioactive natural products. Meanwhile, the n-hexane fraction (EFH) exhibited moderate and selective cytotoxicity toward MCF-7 breast cancer cells. These findings provide a strong foundation for future bioassay-guided fractionation studies aimed at isolating and identifying the active constituents responsible for the observed anticancer effects.

4. Conclusion

The present study investigated the phytochemical composition and selected biological activities of extracts obtained from the aerial parts of *Eupatorium fortunei* Turcz. collected from Tam Dao, Phu Tho Province, Vietnam. The major findings can be summarized as follows:

(1) Phytochemical composition: Preliminary phytochemical screening revealed the presence of five major groups of biologically active secondary metabolites, namely alkaloids, coumarins, flavonoids, terpenoids, and proanthocyanidins. In contrast, cardiac glycosides, saponins, and tannins were not detected under the experimental conditions employed. Thin-layer chromatography (TLC) analysis showed that the n-hexane fraction contained at least ten well-resolved constituents using an n-hexane acetate (5:1, v/v) mobile phase, with aromatic compounds and natural pigments being the predominant components. The ethyl acetate fraction exhibited greater chemical diversity, with at least sixteen constituents optimally separated using a dichloromethane (20:1, v/v) solvent system, characteristic of moderately polar conjugated polycyclic compounds such as flavonoids and phenolic acids.

(2) Antimicrobial activity: Both extract fractions exhibited weak to moderate antimicrobial activity, with MIC values ranging from 100 to 200 μg/mL. The n-hexane fraction (EFH) showed the strongest selective activity against the pathogenic yeast *Candida albicans* (MIC = 100 μg/mL) and inhibited *Bacillus subtilis* and *Fusarium oxysporum* at 200 μg/mL. The ethyl acetate fraction (EFE) demonstrated limited activity (MIC = 200 μg/mL) against *Escherichia coli*, *Fusarium oxysporum*, and *Candida albicans*.

(3) Cytotoxic activity: Among the tested fractions, the ethyl acetate fraction (EFE) exhibited the most promising in vitro cytotoxic and antiproliferative activity, particularly against the human lung cancer cell line A549, with an IC_{50} value of 27.36 ± 1.05 μg/mL, meeting the screening criterion established by the U.S. National Cancer Institute (NCI) for crude plant extracts. The n-hexane fraction (EFH) displayed moderate and selective cytotoxicity toward the human breast cancer cell line MCF-7, with an IC_{50} value of 31.45 ± 1.18 μg/mL.

Collectively, these findings provide important experimental evidence supporting the

pharmacological potential of *Eupatorium fortunei* cultivated in the Tam Dao region. The plant represents a promising source of bioactive compounds for the development of natural products with potential applications in cancer therapy—particularly against lung and breast cancers—as well as in the control of pathogenic microorganisms.

Acknowledgements:

The authors would like to express their sincere gratitude to Tan Trao University for financial and technical support in conducting this study.

Corresponding Author:

Tran Duc Dai[1]*: Faculty of Medicine and Pharmacy, Tan Trao University
E-mail: TDDai@tqu.edu.vn

References:

[1] Do Tat Loi, Medicinal Plants and Medicinal Substances of Vietnam, Hanoi, VietNam: Medical Publishing House, 2004.

[2] Vo Van Chi, "Dictionary of Vietnamese Medicinal Plants," *Hanoi, Vietnam: Medical Publishing House,* vol. 2, 2012.

[3] Do Huy Bich, B. X, "Medicinal Plants and Medicinal Animals of Vietnam," *Hanoi, Vietnam: Science and Technology Publishing House,* vol. 2, 2006.

[4] Jian Qiang Ma, Y. P, "Eupatorium fortunei Turcz.: An updated review on the botany, phytochemistry, pharmacology, and toxicology," *Molecules,* vol. 31, no. 7, p. 1137, 2026.

[5] Rui Dong, S. L, "Diverse thymol analogs from Eupatorium fortunei: Their anti-inflammatory activity and mechanism," *Fitoterapia,* vol. 191, p. 107249, 2016.

[6] Lei Miao, Q. W, "Chemical constituents from Eupatorium fortunei and their anti-inflammatory evaluation by in silico and experimental approaches," *Fitoterapia,* vol. 171, p. 105700, 2023.

[7] Yanqun Li, D. K, "The effect of developmental and environmental factors on secondary metabolites in medicinal plants," *Plant Physiology and Biochemistry,* vol. 148, pp. 80-89, 2020.

[8] Poonam Pant, S. P, "The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review," *Chemistry & Biodiversity,* vol. 18, no. 11, p. e2100345, 2024.

[9] Li Yang, K. S.-X, "Response of plant secondary metabolites to environmental factors," *Molecules,* vol. 23, no. 4, p. 762, 2018.

[10] Ministry of Health of Vietnam, "Vietnamese Pharmacopoeia V," *Hanoi, Vietnam: Medical Publishing House,* 2018.

[11] T. T. An, "Analytical Chemistry," *Hanoi, Vietnam: Medical Publishing House,* vol. 2, pp. 168-212, 2012.

[12] L. Mckane, "Bacterial growth and laboratory cultivation," *Microbiology Essentials and Applications, New York, NY, USA: McGraw-Hill,* vol. 97, p. 335, 1996.

[13] D. A. Vanden Berghe, "Screening methods for antibacterial and antiviral agents from higher plants," *in Methods in Plant Biochemistry,* vol. 6, pp. 47-69, 1991.

[14] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay," *Journal of Immunological Methods,* vol. 65, pp. 55-63, 1983.

[15] Ke Son Phan, P. T, "Dual loading of doxorubicin and magnetic iron oxide into PLA-TPGS nanoparticles: Design, in vitro drug release kinetics, and biological effects on cancer cells," *ChemMedChem,* vol. 16, no. 23, pp. 3615-3625, 2021.

[16] M. K. H. Dvora, "10 - Microbial production of flavonoids and terpenoids," *Woodhead Publishing Series in Food Science, Technology and Nutrition,* pp. 234-261, 2013.

[17] J. B. Harborne, "Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis," *London, UK: Chapman & Hall,* 1972.

[18] G. A. Cordell, "Biodiversity and drug discovery—A symbiotic relationship," *Phytochemistry,* vol. 55, no. 6, pp. 463-480, 2000.

[19] D. S. Seigler, "Plant Secondary Metabolism," *New York, NY, USA: Springer Science & Business Media,* pp. 506-513, 1998.

[20] J. M. Robert D. H. Murray, "The Natural Coumarins: Occurrence. Chemistry and Biochemistry," *Chichester, UK: Wiley,* p. 702, 1982.

[21] H. W. Bladt, "Plant Drug Analysis: A Thin Layer Chromatography Atlas, 2nd ed," *Berlin, Germany: Springer-Verlag,* pp. 305-334, 1996.

[22] J. Gross, "Pigments in Vegetables: Chlorophylls and Carotenoids," *New York, NY, USA: Van Nostrand Reinhold,* pp. 3-25, 1991.

[23] A. Marston, "Thin-layer chromatography with biological detection in phytochemistry," *Journal of Chromatography A,* vol. 1218, no. 19, pp. 2676-2683, 2011.

[24] T. K. Mieczysław, "Thin-Layer Chromatography (TLC) in the Screening of Botanicals–Its Versatile Potential and Selected Applications," *Molecules,* vol. 27, pp. 1-23, 2022'.

[25] S. I. Behbahani, "Applications of ultraviolet visualization in thin-layer chromatography for screening of secondary plant metabolites," *Phytochemical Analysis,* vol. 28, no. 4, pp. 211-219, 2017.

[26] J. B. Harborne, "Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis," *3rd ed. London, UK: Chapman & Hall,* pp. 40-90, 1998.

[27] R. H. D A Scudiero, "Evaluation of a soluable tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines," *Cancer Research,* vol. 48, pp. 4827-4833, 1988.

[28] M. Sainsbury, "The anticancer substance ellipticine and its derivatives," *Synthesis,* vol. 24, no. 7, pp. 437-448, 1977.

[29] I. G. Mohammad Adam Mustapa, "Anti-Breast Cancer Activity of Essential Oil: A Systematic Review," *Applied Sciences,* vol. 12, no. 24, p. 12738, 2022.

[30] A. M.-N. Shaza H. Aly, "Unveiling the pharmacological potential of plant triterpenoids in breast cancer management: an updated review," *Naunyn-Schmiedeberg's Archives of*

Pharmacology, vol. 397, pp. 5571-5596, 2024.

[31] A. G. Linara Gabitova, "Molecular Pathways: Sterols and Receptor Signaling in Cancer," *Clinical Cancer Research,* vol. 20, no. 1, pp. 28-34, 2014.

[32] A. J. T P Tim Cushnie, "Recent advances in understanding the antibacterial and anticancer activities of flavonoids," *Phytochemistry Reviews,* vol. 14, no. 5, pp. 741-759, 2015.