PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF THE ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF MICHELIA TONKINENSIS SEEDS EXTRACTS FROM NORTHERN VIETNAM

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ABSTRACT

Michelia tonkinensis A. Chev., a valuable medicinal and economic tree species widely distributed in the mountainous regions of northern Vietnam, has traditionally been used as both a culinary spice and a folk remedy for musculoskeletal and gastrointestinal disorders. However, information regarding the phytochemical composition and biological activities of its seeds remains limited. This study aimed to investigate the phytochemical constituents, antibacterial activity, and antioxidant potential of *M. tonkinensis* seeds collected from Tuyen Quang Province, Vietnam. Seed powder was extracted with 70% ethanol using ultrasound-assisted extraction, followed by liquid–liquid partitioning to obtain *n*-hexane (MTH) and ethyl acetate (MTE) fractions. Preliminary phytochemical screening was performed according to the Vietnamese Pharmacopoeia V. Antibacterial activity was evaluated using the broth microdilution method in 96-well microplates against selected bacterial and fungal strains, while antioxidant activity was assessed using the DPPH radical scavenging assay.

Phytochemical analysis revealed the presence of coumarins, flavonoids, proanthocyanidins, and terpenoids in the seed extracts. The MTH fraction exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* with a minimum inhibitory concentration (MIC) of 200 µg/mL. The MTE fraction showed inhibitory activity against *Saccharomyces cerevisiae* at the same MIC value. In the DPPH assay, MTH demonstrated stronger antioxidant activity (SC_{50} = 44.97 ± 1.29 µg/mL) than MTE (SC_{50} = 59.25 ± 1.68 µg/mL), although both were less active than the positive control, ascorbic acid (SC_{50} = 12.31 ± 0.21 µg/mL). These findings indicate that *M. tonkinensis* seeds are a promising source of bioactive phytochemicals with notable antibacterial and antioxidant properties. The study provides a scientific basis for future isolation

and characterization of active compounds and supports the potential development of seed-derived functional foods and phytopharmaceutical products.

1. INTRODUCTION

Natural products derived from medicinal plants continue to play a pivotal role in the discovery of bioactive compounds for pharmaceutical, nutraceutical, and functional food applications. Plant secondary metabolites, including flavonoids, terpenoids, alkaloids, coumarins, and phenolic compounds, have attracted significant scientific interest due to their diverse biological activities, particularly antioxidant, antimicrobial, anti-inflammatory, and anticancer effects [1], [2].

In recent decades, oxidative stress and antimicrobial resistance have emerged as major global health concerns. Excessive production of reactive oxygen species (ROS) contributes to cellular damage and is implicated in the pathogenesis of numerous chronic diseases, including cardiovascular disorders, diabetes mellitus, neurodegenerative diseases, and cancer [3]. At the same time, the increasing prevalence of multidrug-resistant microorganisms has reduced the effectiveness of conventional antibiotics and stimulated the search for alternative antimicrobial agents from natural sources [4]. Consequently, medicinal plants possessing both antioxidant and antimicrobial properties have become promising candidates for the development of novel therapeutic agents and health-promoting products.

The family Magnoliaceae comprises numerous medicinally important species distributed throughout tropical and subtropical regions of Asia. Members of this family are known to produce a wide range of biologically active metabolites, including sesquiterpenes, neolignans, flavonoids, alkaloids, and phenolic compounds. Previous studies have demonstrated that extracts and essential oils obtained from Magnoliaceae species exhibit remarkable antioxidant, antibacterial, antifungal, anti-inflammatory, and cytotoxic activities [1], [5].

Among these species, *Michelia tonkinensis* A. Chev., commonly known as "Giổi ăn hạt", is an economically and medicinally valuable tree endemic to northern Vietnam. The species is widely distributed in mountainous provinces, including Tuyen Quang, Son La, Yen Bai, and Lai Chau. Its seeds are traditionally used as a culinary spice because of their characteristic aroma and pungent flavor, while different parts of the plant have been employed in Vietnamese folk medicine for the treatment of fever, digestive disorders, musculoskeletal pain, and infectious diseases [6]. Owing to its economic value, *M. tonkinensis* has also been cultivated for timber production and agroforestry development in mountainous regions.

Previous phytochemical investigations of *Michelia* species have identified various classes of secondary metabolites, particularly terpenoids and phenolic compounds, which are frequently associated with antioxidant and antimicrobial activities [7], [5]. Furthermore, essential oils isolated from *M. tonkinensis* have been reported to contain α-pinene, β-phellandrene, β-caryophyllene, linalool, and other volatile constituents with potential biological activities [8], [9]. However, most

published studies have focused primarily on the chemical composition of essential oils, whereas information regarding the phytochemical composition and biological activities of solvent-partitioned seed extracts remains limited.

In particular, no comprehensive study has simultaneously investigated the phytochemical constituents, antibacterial activity, and antioxidant potential of *M. tonkinensis* seed extracts collected from Tuyen Quang Province, one of the important natural distribution areas of the species in Vietnam. Therefore, the present study was conducted to (i) perform preliminary phytochemical screening of *M. tonkinensis* seed extracts, (ii) evaluate their antibacterial activity against selected bacterial and fungal strains, and (iii) assess their antioxidant potential using the DPPH radical scavenging assay. The findings are expected to provide scientific evidence supporting the traditional use of this species and contribute to future studies on bioactive compound isolation and the development of functional food and phytopharmaceutical products.

2. RELATED WORKS

Research on the genus *Michelia* has attracted increasing attention because of its rich phytochemical diversity and wide range of biological activities. [1] reviewed phytochemical and pharmacological studies of *Michelia* species and reported the occurrence of numerous secondary metabolites, including alkaloids, flavonoids, terpenoids, lignans, steroids, and phenolic compounds. These metabolites have been associated with antioxidant, antimicrobial, antiplasmodial, anti-inflammatory, and anticancer properties, highlighting the pharmaceutical potential of the genus [2].

Several studies have demonstrated that antioxidant activity in medicinal plants is closely related to the presence of flavonoids and phenolic compounds. Flavonoids possess strong free-radical scavenging activity through electron donation, hydrogen atom transfer, and metal-chelating mechanisms [2]. Similarly, Shahidi and Ambigaipalan emphasized that phenolic-rich plant extracts represent valuable natural antioxidants for pharmaceutical and food applications because they can prevent oxidative deterioration and reduce oxidative stress-related damage [10].

The antimicrobial properties of plant-derived secondary metabolites have also been extensively investigated. Cushnie and Lamb reported that flavonoids exert antibacterial effects through multiple mechanisms, including disruption of microbial membranes, inhibition of nucleic acid synthesis, and interference with energy metabolism [11]. Likewise, terpenoids are capable of altering membrane permeability and impairing essential physiological processes in microorganisms, thereby contributing significantly to the antimicrobial activity of plant extracts and essential oils [12].

Within the genus *Michelia*, *Michelia champaca* has been one of the most extensively studied species. Parimi and Kolli reported that flower extracts of *M. champaca* exhibited both antioxidant and antibacterial activities. The authors observed that ethyl acetate extracts demonstrated stronger DPPH radical scavenging activity than non-polar fractions, suggesting that moderately polar compounds contribute substantially to antioxidant effects. Similar findings have been reported for other species within Magnoliaceae, where the coexistence of flavonoids, phenolics, and terpenoids often results in synergistic biological activities [13].

Recent investigations have focused on the chemical composition and biological activities of essential oils from Magnoliaceae species. Yang et al. analyzed essential oils from five

representative species and identified sesquiterpenes and monoterpenes as the dominant constituents. The study demonstrated significant antibacterial and antioxidant activities, which were attributed primarily to compounds such as β-caryophyllene, nerolidol, and α-pinene. These findings further support the role of terpenoid-rich extracts as potential natural antimicrobial and antioxidant agents [5].

Studies specifically targeting *Michelia tonkinensis* remain relatively scarce. Do et al. characterized the essential oil composition of several Magnoliaceae species cultivated in Vietnam and reported that *M. tonkinensis* contained substantial amounts of monoterpenes and sesquiterpenes. More recently, Tran et al. investigated essential oils obtained from leaves, twigs, fruit bark, and seeds of *M. tonkinensis*. Their results confirmed the presence of bioactive volatile constituents and demonstrated antimicrobial activity against selected microorganisms [9], [8].

Despite these advances, several important knowledge gaps remain. First, most available studies have concentrated on volatile constituents and essential oils rather than solvent extracts. Second, the occurrence of non-volatile phytochemicals such as flavonoids, coumarins, and proanthocyanidins in *M. tonkinensis* seeds has received limited attention. Third, information regarding the biological activities of solvent-partitioned seed extracts from natural populations growing in Tuyen Quang Province is lacking. Addressing these gaps is essential for understanding the medicinal potential of this species and identifying promising sources of bioactive compounds for future pharmaceutical and functional food applications.

Therefore, the present study expands current knowledge by providing a comprehensive assessment of phytochemical constituents and evaluating the antibacterial and antioxidant activities of n-hexane and ethyl acetate extracts obtained from *M. tonkinensis* seeds collected in Tuyen Quang Province, Vietnam.

3. PROPOSED METHODOLOGY

3.1. Plant Material and Taxonomic Identification

Seeds of *Michelia tonkinensis* A. Chev. were collected from Yen Son Commune, Tuyen Quang Province, Vietnam, in October 2025. Botanical identification was performed using voucher specimens consisting of branches bearing leaves, flowers, and fruits. The species was authenticated as *Michelia tonkinensis* A. Chev. by Mr. Nghiem Duc Trong, M.Sc., Faculty of Pharmacy, Hanoi University of Pharmacy, Vietnam.

Figure 1. Cluster of fruits and seeds of *Michelia tonkinensis*.

A voucher specimen was deposited at the Pharmaceutical Practice Center, Faculty of Medicine and Pharmacy, Tan Trao University, Vietnam, where the corresponding seed samples were also preserved for future reference and subsequent analyses. The scientific nomenclature of the species follows currently accepted taxonomic classifications for the family Magnoliaceae.

3.2. Materials and Methods

3.2.1. Instruments, Chemicals, and Extraction Equipment

Instruments and Laboratory Equipment: The experimental work was carried out using standard laboratory instruments, including an analytical balance, precision balance, drying oven, laboratory dryer, electric heating plate, water bath, rotary vacuum evaporator, 20 L ultrasonic extraction bath, essential oil distillation apparatus, optical microscope, microtome blades, ultraviolet lamp (254 and 365 nm) for thin-layer chromatography (TLC) visualization, reagent sprayers, silica gel TLC plates, separatory funnels, Erlenmeyer flasks, glass beakers, test tubes, pipettes, porcelain mortars, glass rods, and filter papers.

Chemicals and Reagents: Analytical-grade solvents and reagents were used throughout the study, including methanol, ethanol (96%, v/v), *n*-hexane, ethyl acetate, chloroform, formic acid, ethanol (70%, v/v), hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), acetic acid (5%, v/v), sodium hydroxide (NaOH, 10%, w/v), ferric chloride ($FeCl_3$, 5%, w/v), gelatin solution (1%,

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w/v), Mayer's reagent, Bouchardat's reagent (I_2/KI), sodium hypochlorite solution, carmine red, and methylene blue.

All chemicals and solvents employed in this study were of analytical grade and used without further purification.

3.2.2. Extraction and Fractionation of *Michelia tonkinensis* Seed Extracts

The collected seeds of *Michelia tonkinensis* were dried at a controlled temperature until a constant weight was achieved and subsequently ground into a fine powder. The powdered material was stored in airtight containers for further phytochemical and biological analyses.

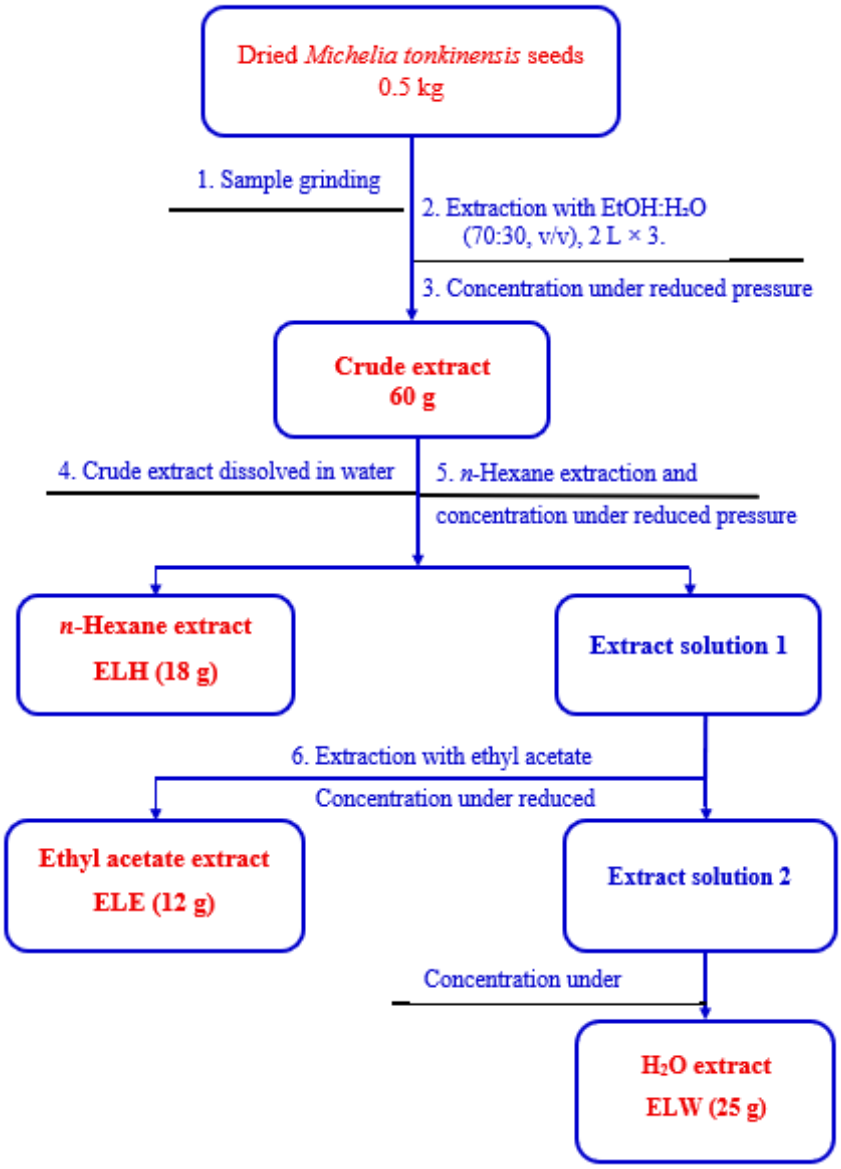

Figure 2. Schematic representation of the extraction and fractionation procedure for the aerial parts of *Michelia tonkinensis* seeds.

Briefly, 500 g of dried seed powder was extracted with 70% aqueous ethanol (EtOH:H_2O, 70:30, v/v) using an ultrasound-assisted extraction (UAE) method. The plant material was immersed in 2 L of extraction solvent in a 7 L glass vessel and subjected to ultrasonic treatment in a 20 L ultrasonic bath at 50°C for 24 h. After extraction, the mixture was filtered through Whatman

filter paper to remove solid residues. The extraction process was repeated three times under identical conditions to ensure exhaustive extraction of phytochemical constituents. The filtrates were combined to obtain the crude hydroethanolic extract.

The pooled extract was concentrated under reduced pressure using a rotary vacuum evaporator at 50°C to remove the solvent and yield 60 g of crude ethanol extract. The concentrated extract was stored at 4°C until further use.

For solvent partitioning, 60 g of the crude extract was suspended in 500 mL of distilled water and successively fractionated with solvents of increasing polarity. Liquid–liquid extraction was first performed using n-hexane, followed by ethyl acetate. The organic phases were collected separately and concentrated under reduced pressure to afford 18 g of the *n*-hexane fraction (MTH) and 12 g of the ethyl acetate fraction (MTE). The remaining aqueous phase yielded 25 g of the water fraction (MTW).

The obtained fractions were subsequently subjected to preliminary phytochemical screening, antibacterial assays, and antioxidant activity evaluation. The extraction and fractionation procedure was adapted from commonly employed protocols for the isolation of bioactive compounds from medicinal plants.

3.2.3. 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 [14]. 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.

3.2.4. 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 and McKane and Kandel [15], [16].

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 (Mckane, 1996), (Vanden Berghe,

1991).

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.

3.2.5. Antioxidant Activity Assessment

The antioxidant activity of *Michelia tonkinensis* A. Chev. seed essential oil and solvent fractions was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the method originally described by Brand-Williams et al, and subsequently modified by Kumar et al, to suit the experimental conditions employed in this study [17], [18].

Briefly, the essential oil and extract fractions obtained from *M. tonkinensis* seeds were dissolved in dimethyl sulfoxide (DMSO) and serially diluted with ethanol (EtOH) to obtain a range of test concentrations. A DPPH stock solution was freshly prepared in ethanol at a concentration of 0.1 mM.

For the assay, 190 µL of 0.1 mM DPPH solution was mixed with 10 µL of each sample solution at different concentrations in individual wells of a 96-well microplate. The reaction mixtures were incubated at 37°C in the dark for 30 min to allow complete interaction between antioxidants and DPPH radicals. Ascorbic acid was used as a positive control due to its well-established free radical scavenging activity. The negative control (blank) consisted of 0.1 mM DPPH solution prepared in an ethanol–DMSO mixture without the addition of sample.

Following incubation, the absorbance of each reaction mixture was measured at 515 nm using a microplate spectrophotometer. The DPPH radical scavenging activity (SC%) was calculated according to Equation (1):

$$SC\ (\%) = [(ODc - ODs)/ODc] \times 100\%$$

where (SC%) represents the percentage of DPPH radical scavenging activity, ODs is the absorbance of the sample, and ODc is the absorbance of the negative control.

All experiments were performed in triplicate to ensure the reliability and reproducibility of the results. The SC_{50} value (equivalent to IC_{50} was determined by linear regression analysis using the equation $y = ax + b$, which describes the relationship between sample concentration (C) and scavenging activity (SC%). SC_{50} was defined as the concentration required to neutralize 50% of DPPH radicals. Lower SC_{50} values indicate stronger antioxidant activity and greater free radical scavenging capacity.

4. RESEARCH RESULTS

4.1. Extraction yield and preparation of crude extracts

From 0.5 kg of dried *Michelia tonkinensis* seed powder, ultrasound-assisted extraction afforded 60 g of crude extract. Subsequent liquid–liquid partitioning yielded 18 g of n-hexane fraction (MTH), 12 g of ethyl acetate fraction (MTE), and 25 g of aqueous fraction (MTW).**4.2. Preliminary qualitative phytochemical analysis**

The preliminary phytochemical screening of *Michelia tonkinensis* extracts revealed a distinct distribution of secondary metabolite classes, as summarized in **Table 1.** The results were interpreted based on standard colorimetric and precipitation reactions characteristic of each phytochemical group, which are widely used in qualitative phytochemical investigations of plant materials [19], [20].

The absence of alkaloids was confirmed by negative reactions with Dragendorff's, Mayer's, and Bouchardat's reagents. These reagents are commonly employed for alkaloid detection due to their ability to form insoluble complexes with nitrogen-containing bases (Harborne, 1998). Similarly, glycosides, including cardiac glycosides, were not detected, as evidenced by negative Keller–Killiani and Xanthydrol tests, indicating that this class of compounds is either absent or present below detectable levels in the tested extracts.

In contrast, coumarins were strongly detected (+++) through the characteristic alkaline hydrolysis reaction followed by acidification, which typically results in fluorescence or color development due to lactone ring opening and re-lactonization processes. This suggests that *M. tonkinensis* is a rich source of coumarin-type metabolites, which are known for diverse biological activities, including anti-inflammatory and antimicrobial properties [20].

Flavonoids also showed a strong positive reaction (+++) with ferric chloride, indicating the presence of phenolic hydroxyl groups capable of forming colored complexes with Fe^{3+} ions. This result is consistent with the widespread occurrence of flavonoids in higher plants and their role as major antioxidant constituents [19], [21].

Proanthocyanidins were detected at a weak positive level (+), suggesting a relatively low abundance of condensed tannins. Anthocyanins showed an ambiguous response (-/+), which may be attributed to their instability under extraction or pH conditions, as these compounds are known to undergo structural transformations depending on environmental pH [22].

Notably, tannins were not detected using both gelatin–NaCl and vanillin–HCl assays. This suggests either the absence of hydrolysable and condensed tannins or their presence below detection thresholds. Saponins were also not observed, as indicated by the absence of stable foam formation, implying low surfactant-type glycosides in the extracts.

A strong positive reaction (+++) was observed for terpenoids using the chloroform–concentrated sulfuric acid test, indicating the presence of steroidal or triterpenoid constituents. The formation of characteristic color changes in this test is widely accepted as evidence for terpenoid skeletons, which are common in the Magnoliaceae family and are associated with various pharmacological properties [19], [23].

Table 1. Preliminary phytochemical screening of chemical constituents in *Michelia tonkinensis* extracts.

No	Substance group	Qualitative reaction	Result
1	Alkaloid	Reaction with Bouchardat reagent (Iod/Kali iodid)	-
		Reaction with Mayer (Kali tetraiodomercurat)	-
		Reaction with Dragendorff (Kali iodobismuthat)	-
2	Coumarin	Reaction with 10% NaOH and acidification with HCl	+++
3	Glycosid tim	Reaction with Keller-Kiliani	-
		Reaction with Xanthydrol	-
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%-NaCl 10%	-

		Reaction with Vanilin - HCl	-
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.

Overall, the phytochemical profile of *M. tonkinensis* is dominated by coumarins, flavonoids, and terpenoids, while alkaloids, tannins, glycosides, and saponins appear to be absent or present in trace amounts. These findings provide a chemical basis for further isolation and characterization of bioactive constituents from this species.

4.3. Antibacterial activity

The minimum inhibitory concentration (MIC) results of two extracts obtained from *Michelia tonkinensis* seeds (MTH: *n*-hexane extract; MTE: ethyl acetate extract) indicate overall weak to very weak antimicrobial activity, with MIC values ranging from 200 µg/mL to > 200 µg/mL against most tested microorganisms **Table 2, Table 3**. According to commonly accepted criteria for crude plant extracts, MIC values above 100 µg/mL generally indicate weak activity, while values above 1000 µg/mL are considered inactive or negligible. Therefore, the observed MIC of 200 µg/mL reflects only limited inhibitory potential [24].

4.3.1. Comparison between extract types

The *n*-hexane extract (MTH) exhibited a broader antimicrobial spectrum compared to the ethyl acetate extract (MTE). MTH inhibited *Escherichia coli*, *Staphylococcus aureus*, and *C. albicans* at MIC = 200 µg/mL, whereas no activity was observed against *Pseudomonas aeruginosa*, *B. subtillis, A.niger*, *F. oxysporum*, *S.cerevisiae* and most yeast strains (MIC > 200 µg/mL). In contrast, MTE showed negligible antimicrobial activity, with inhibition observed only against *Saccharomyces cerevisiae* (MIC = 200 µg/mL), while all other tested microorganisms exhibited MIC values > 200 µg/mL.

This difference suggests that non-polar compounds extracted in *n*-hexane may contribute more significantly to the observed bioactivity. Such compounds typically include terpenoids, lipophilic metabolites, and other hydrophobic constituents capable of interacting with microbial cell membranes [25].

Table 2. Antibacterial activity (MIC) of MTH and MTE extracts.

No	Sample	Highest test sample concentration (µg/mL)	Minimum inhibitory concentration (MIC, µg/mL)			
			Gram-negative bacteria		Gram-positive bacteria	
			E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*
1	MTH	200	200	> 200	> 200	200
2	MTE	200	> 200	> 200	> 200	> 200
	Tetracycline (positive control)		4	32	1	1
	Nystatin (positive control)		-	-	-	-

"–" indicates no activity or not tested.

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4.3.2. Susceptibility patterns among microbial groups

Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, showed higher resistance compared to Gram-positive bacteria. This observation is consistent with the structural characteristics of Gram-negative bacteria, which possess an outer membrane rich in lipopolysaccharides that restricts the penetration of antimicrobial compounds [26], [27]. In contrast, Gram-positive bacteria such as *S. aureus* were moderately susceptible, with MIC values of 200 µg/mL.

Regarding fungi, both extracts exhibited limited antifungal activity. MTH inhibited *C. albicans*, while MTE showed activity against *S. cerevisiae*. No significant inhibition was observed against *F. oxysporum* or *A. niger*.

4.3.3. Comparison with positive controls

The positive control tetracycline demonstrated strong antibacterial activity, with MIC values ranging from 1 to 32 µg/mL, while nystatin exhibited antifungal activity with MIC values of 4–8 µg/mL, consistent with established susceptibility standards [27]. In comparison, both plant extracts were substantially less potent than standard antibiotics, indicating that they are still at an early stage of bioactivity potential.

Table 3. Antifungal activity (MIC) of MTH and MTE extracts.

No	Sample	Highest test sample concentration (µg/mL)	Minimum inhibitory concentration (MIC, µg/mL)			
			Molds		Yeasts	
			A.niger	*F.oxysporum*	*S.cerevisiae*	*C.albicans*
1	MTH	200	> 200	> 200	> 200	200
2	MTE	200	> 200	> 200	200	> 200
	Tetracycline (positive control)		-	-	-	-
	Nystatin (positive control)		8	8	4	4

"–" indicates no activity or not tested.

4.3.4. Phytochemical and biological implications

The weak antimicrobial activity observed may be attributed to low concentrations of active constituents in crude extracts or the absence of compound purification. Species within the genus *Michelia* are known to contain alkaloids, sesquiterpenes, and phenolic compounds with potential antimicrobial properties; however, their efficacy strongly depends on extraction methods and solvent polarity (Cowan, 1999). The stronger activity of MTH compared to MTE further suggests that non-polar constituents may play a key role in the antimicrobial effects observed in this study.

4.3.5. Antioxidant activity evaluated by the DPPH radical scavenging assay

The antioxidant activity of *Michelia tonkinensis* seed extracts was evaluated using the DPPH radical scavenging assay, and the results are presented in **Table 4.** Both extracts exhibited noticeable free-radical scavenging activity, although their effectiveness was lower than that of the positive control (ascorbic acid).

Table 4. DPPH radical scavenging activity and SC_{50} values of *Michelia tonkinensis* seed extracts.

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No	Sample	Radical scavenging capacity (SC, %)	SC_{50} (μg/mL)
	Positive control (ascorbic acid)	83.23 ± 0.50	12.31 ± 0.21
	Negative control (DPPH/EtOH + DMSO)	0.0 ± 0.00	-
1	MTH	68.78 ± 2.75	44.97 ± 1.29
2	MTE	65.16 ± 2.34	59.25 ± 1.68

Ascorbic acid showed a scavenging capacity (SC) of 83.23 ± 0.50% and an SC_{50} value of 12.31 ± 0.21 µg/mL, confirming its strong antioxidant potential. In contrast, the *n*-hexane extract (MTH) exhibited a scavenging capacity of 68.78 ± 2.75% with an SC_{50} value of 44.97 ± 1.29 µg/mL, whereas the ethyl acetate extract (MTE) showed a scavenging capacity of 65.16 ± 2.34% and an SC_{50} value of 59.25 ± 1.68 µg/mL. Since a lower SC_{50} value indicates stronger antioxidant activity, MTH demonstrated a higher free-radical scavenging capacity than MTE.

The observed antioxidant activity may be attributed to the presence of bioactive compounds capable of donating hydrogen atoms or electrons to neutralize DPPH radicals. The stronger activity of MTH compared with MTE suggests that non-polar or moderately non-polar antioxidant constituents may be concentrated in the n-hexane fraction. Previous phytochemical investigations of *Michelia* species have reported the occurrence of lignans, terpenoids, essential oil components, and phenolic derivatives, many of which possess significant antioxidant properties [28].

Although both extracts exhibited lower activity than ascorbic acid, their SC values exceeding 65% indicate a considerable radical-scavenging potential. The difference between MTH and MTE may also reflect variations in the polarity-dependent extraction of antioxidant constituents. Solvent polarity strongly influences the recovery of phytochemicals, resulting in differences in antioxidant effectiveness among extracts obtained from the same plant material [29].

The results obtained in this study are consistent with previous reports demonstrating antioxidant activities in extracts from members of the family Magnoliaceae. Several species within this family have been shown to possess moderate to strong DPPH radical scavenging activity due to the presence of phenolic compounds and oxygenated terpenoids [30]. Therefore, the antioxidant activity observed in *M. tonkinensis* seed extracts may contribute to the biological and pharmacological value of this species and support its potential use as a natural source of antioxidant agents.

5. CONCLUSION AND FUTURE DEVELOPMENT

The present study provides a comprehensive evaluation of the phytochemical composition, antibacterial activity, and antioxidant potential of *n*-hexane (MTH) and ethyl acetate (MTE) seed extracts of *Michelia tonkinensis* collected from Tuyen Quang Province, Vietnam. The preliminary phytochemical screening revealed that the extracts were rich in coumarins, flavonoids, and terpenoids, while alkaloids, glycosides, tannins, and saponins were not detected or were present at negligible levels. This chemical profile suggests that moderately polar and non-polar secondary metabolites are the major constituents of the seed extracts.

The antimicrobial assay demonstrated that both extracts exhibited weak to moderate antibacterial activity, with MTH showing slightly broader inhibitory effects compared to MTE. However, both extracts were generally ineffective against Gram-negative bacteria and most fungal strains, as indicated by relatively high MIC values (≥ 200 µg/mL). These results suggest that crude seed extracts of *M. tonkinensis* possess limited direct antimicrobial potency in their unrefined form.

In contrast, the antioxidant evaluation using the DPPH assay revealed more promising results. Both extracts exhibited notable radical scavenging activity, with SC% values exceeding 65%, although lower than that of ascorbic acid. The n-hexane fraction (MTH) demonstrated stronger antioxidant potential (SC_{50} = 44.97 ± 1.29 µg/mL) compared to the ethyl acetate fraction (MTE) (SC_{50} = 59.25 ± 1.68 µg/mL). These findings indicate that non-polar constituents may play a more important role in the antioxidant capacity of the seeds.

Overall, the results suggest that *M. tonkinensis* seeds represent a potential natural source of bioactive compounds, particularly with antioxidant properties, although their antimicrobial activity remains limited at the crude extract level.

Future development: Future studies should focus on bioassay-guided fractionation and advanced chromatographic techniques (e.g., GC–MS, LC–MS/MS, and NMR) to isolate and identify the bioactive constituents responsible for the observed antioxidant and antimicrobial activities. In addition, further antioxidant assays (ABTS, FRAP, and ORAC) and in vivo evaluations are necessary to better elucidate the mechanism of action and pharmacological potential of *Michelia tonkinensis* seed extracts.

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