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**CHEMICAL COMPOSITION ANALYSIS AND
EVALUATION OF THE GROWTH INHIBITORY
POTENTIAL AGAINST CYANOBACTERIUM
Microcystis aeruginosa BY *Chrysanthemum indicum* AND
*Acacia mangium***

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INTRODUCTION

The outbreak of cyanobacteria, particularly *Microcystis aeruginosa*, has become a serious concern for freshwater ecosystems worldwide. The excessive proliferation of cyanobacteria not only disrupts aquatic ecosystems but also poses significant threats to human health due to their ability to produce cyanotoxins. Current control methods for *Microcystis aeruginosa* mainly rely on chemical, physical, and biological approaches. However, these methods face limitations such as secondary pollution, high costs, or lack of long-term sustainability. Consequently, the search for environmentally friendly natural compounds capable of inhibiting cyanobacterial growth has become an emerging research direction.

Among potential plant sources, *Chrysanthemum indicum* and *Acacia mangium* are known for their diverse chemical compositions, particularly polyphenols, flavonoids, alkaloids, and terpenoids, many of which exhibit notable biological activities. Previous studies have shown that plant-derived compounds can inhibit cyanobacterial growth through various mechanisms, including membrane disruption, photosynthesis inhibition, and interference with growth cycles. However, detailed investigations into the chemical composition and anti-*Microcystis aeruginosa* activity of these two plant species remain limited.

Based on the above rationale, this doctoral dissertation titled **“Chemical Composition Analysis and Evaluation of the Growth Inhibitory Potential Against Cyanobacterium *Microcystis aeruginosa* by *Chrysanthemum indicum* and *Acacia mangium*”** was conducted to identify bioactive compounds and assess their

inhibitory effects on cyanobacteria. The findings of this dissertation not only contribute to the scientific understanding of the chemical constituents of these plants but also offer promising potential for developing natural compounds for sustainable and eco-friendly cyanobacterial control.

Objectives of the Dissertation:

The dissertation aims to isolate and characterize chemical constituents from the extracts of *Chrysanthemum indicum* flowers and *Acacia mangium* leaves, and to evaluate the growth inhibitory activity of these compounds and extracts against *Microcystis aeruginosa*, thereby contributing to the development of environmentally sustainable cyanobacterial control strategies.

Main Contents of the Dissertation:

1. Investigation of the chemical constituents of *Chrysanthemum indicum* and *Acacia mangium*.
2. Evaluation of the inhibitory activity of plant extracts, isolated compounds, and extract mixtures from *Chrysanthemum indicum* and *Acacia mangium* against *Microcystis aeruginosa*.

CHAPTER 1. INTRODUCTION

1.1. Overview of *Chrysanthemum indicum*

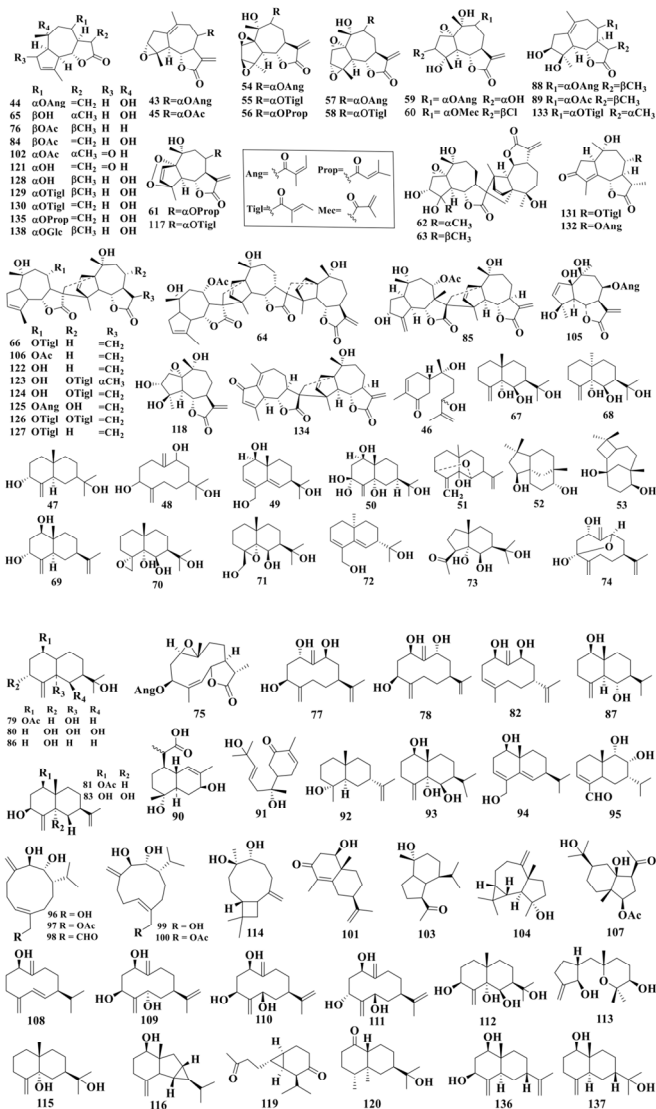
1.1.1. *Taxonomy, Morphology, and Distribution*

Chrysanthemum indicum L., commonly known as wild chrysanthemum, belongs to the family Asteraceae. It is a perennial herbaceous plant that typically grows to a height of 0.25 to 1 meter. The plant has erect or creeping stems, branching, and sparsely hairy. The leaves are alternate, ovate to lanceolate in shape, with serrated margins and a thin hairy layer. The flowers are yellow, forming capitula (head-type inflorescences) surrounded by several rows of involucral bracts. The fruit is an achene, small in size [1]. This species is widely distributed across Asia, particularly in China, Japan, Korea, Vietnam, and India. It typically grows in grasslands, hillsides, riverbanks, paddy fields, and saline or moist coastal areas, at elevations ranging from 100 to 2,900 meters [1].

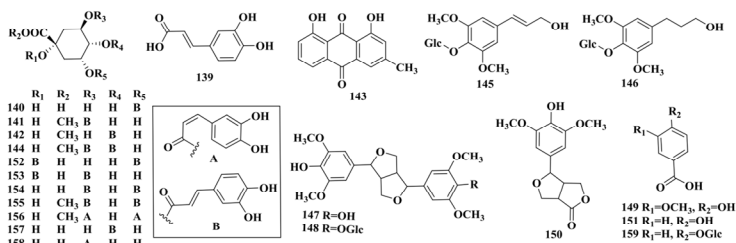
1.1.2. *Chemical constituents of Chrysanthemum indicum*

More than 100 natural compounds have been isolated from *Chrysanthemum indicum* [2]. The major classes of active compounds include flavonoids, terpenoids, phenylpropanoids, phenolic acids, polyacetylenes, and several other groups of secondary metabolites.

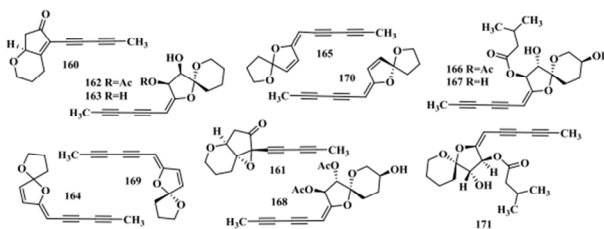
1.1.2.2. Terpenoids



1.1.2.3. Các hợp chất phenylpropanoid và phenolic acid



1.1.2.4. Polyacetylenes



1.1.3. Bioactivities of *Chrysanthemum indicum*

1.1.3.1. Antioxidant

1.1.3.2. Anti-inflammatory activity

1.1.3.3. Anticancer activity

1.1.3.4. Antibacterial and antiviral activities

1.2. Overview of the genus *Acacia* and the species *Acacia mangium*

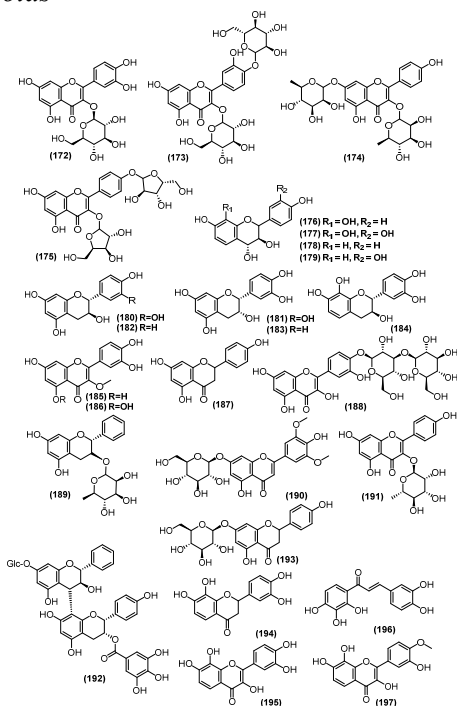
1.2.1. Taxonomy, morphology and distribution of *Acacia mangium*

The genus *Acacia*, belonging to the family Fabaceae and the subfamily Mimosoideae, comprises approximately 1,350 species that are widely distributed across the globe. *Acacia mangium*, commonly

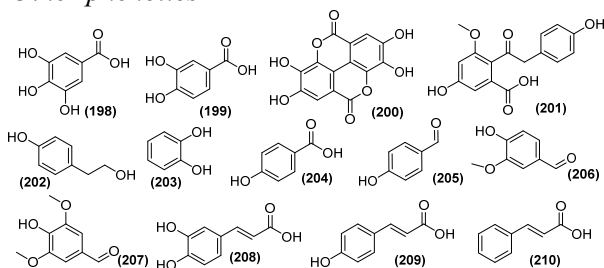
known as mangium or black wattle, is one of the most important species within this genus.

1.2.2. Chemical constituents of the genus *Acacia* and the species *Acacia mangium*

1.2.2.1. Flavonoids



1.2.2.1. Other phenolics



1.2.3. Biological activities of *Acacia mangium* and the genus *Acacia*

- 1.2.3.1. Antioxidant activity*
- 1.2.3.2. Antibacterial and antifungal activities*
- 1.2.3.3. Anti-inflammatory and cytotoxic activities against cancer cells*

1.3. Overview of the cyanobacterium *Microcystis aeruginosa*

1.3.1. Biological characteristics of *Microcystis aeruginosa*

Microcystis aeruginosa is a common cyanobacterium found in freshwater environments, particularly in nutrient-rich waters. It exhibits strong growth in phosphorus-rich conditions, increasing the risk of harmful algal blooms (HABs), which negatively affect water quality and ecosystems[66].

1.3.1.1. Morphology and cellular structure

1.3.1.2. Toxin production and survival strategies

1.3.1.3. Genetic plasticity and environmental adaptability

1.3.2. Environmental and health impacts of *Microcystis aeruginosa*

1.3.2.1. Impacts on the environment

1.3.2.2. Impacts on human health

1.3.3. Methods for controlling *Microcystis aeruginosa* in the environment

Controlling *Microcystis aeruginosa* in aquatic environments poses a significant challenge due to its rapid growth and toxin production. Effective control strategies that minimize ecological disruption are generally categorized into three main approaches: physical, chemical, and biological methods.

1.3.3.1. Physical methods

1.3.3.2. Biological methods

1.3.3.3. Chemical methods

1.3.3.4. Methods using natural-derived agents

1.3.4. Research status on *Microcystis aeruginosa* and control methods in Vietnam

CHAPTER II. EXPERIMENTAL METHODS AND RESULTS

2.1. Research subjects

Flower samples of *Chrysanthemum indicum* were collected in December 2021, during the peak blooming period (Figure 2.1), in Hung Yen. Leaf samples of *Acacia mangium* were collected in June 2020 in Thach That, Hanoi. All plant samples were identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. The specimens are preserved at the Institute of Chemistry of Natural Compounds and the Center for High Technology Research and Development, Vietnam Academy of Science and Technology.

2.2. Research methods

2.2.1. Materials and equipment

2.2.1.1. Equipment, tools, and reagents for compound isolation and structural elucidation

- Precoated TLC plates (Silica gel 60 F254, Merck).
- Silica gel 60 (0.040–0.063 mm, 240–430 mesh ASTM), LiChroprep® RP-18 (40–63 µm), and Diaion HP-20 (all from Merck, Germany).
- NMR spectroscopy: ¹H and ¹³C NMR spectra were recorded using Bruker Avance 500 MHz and 600 MHz spectrometers with tetramethylsilane (TMS) as internal standard, at the Institute of Chemistry, VAST.
- HR-ESI-MS: Agilent 6530 iFunnel Q-TOF LC/MS at the Institute of Marine Biochemistry.
- ESI-MS: Thermo LCQ Fleet LC/MS at the Center for High Technology Research and Development, VAST.
- Optical rotation: JASCO P-2000 polarimeter, Institute of Marine Biochemistry.
- HPLC system: Thermo Ultimate 3000 with DAD detector.
- Preparative chromatography column: YMC ODS-A 250×20 mm, 5 µm.
- Extraction and isolation equipment: ultrasonic bath (Daihan Scientific), rotary evaporator (Buchi R300), automatic fraction collector (EYELA DC-1200), dual-wavelength UV lamp (254/365

nm), chromatography columns, developing chambers, and other laboratory tools.

- Chemicals and reagents: methanol, n-hexane, ethyl acetate, dichloromethane, acetone, cerium sulfate reagent, vanillin reagent, 10% H₂SO₄.

2.2.1.2. Equipment, tools, and reagents for bioactivity assays

- Microplate reader (BioTek Synergy HTX, Agilent, USA).
- Water bath with temperature control.
- Pre-treated 96-well plates (SPL Life Sciences, Korea).
- Micropipettes and multichannel pipettes; pipette tips (Isolab, Germany).
- Eppendorf tubes (1.5 and 2.0 mL).

2.3. Experimental methods

2.3.1. Methods for natural compound isolation

The isolation methods used include:

- Thin-layer chromatography (TLC).
- Column chromatography (CC) with common adsorbents such as silica gel, RP-C18, Sephadex LH-20, and Diaion HP-20.
- Preparative high-performance liquid chromatography (prep-HPLC).
- Analytical high-performance liquid chromatography (HPLC).

2.3.2. Methods for structural elucidation of compounds

The chemical structures of the isolated compounds were determined using modern physicochemical techniques, including nuclear magnetic resonance (NMR), mass spectrometry (ESI-MS), and ultraviolet-visible spectroscopy (UV-Vis).

2.3.3. Optimization method for extract blending ratios

Extract mixtures included three types of extracts from *Eupatorium fortunei* (Ef), *Acacia mangium* (Am), and *Chrysanthemum indicum* (Ci). The blending ratios were determined using a Simplex Lattice Design (SLD) model implemented in Design Expert 12.0 (Stat-Ease, Inc., Minneapolis, USA). The proportion of each extract in the mixture ranged from 5% to 90% by weight.

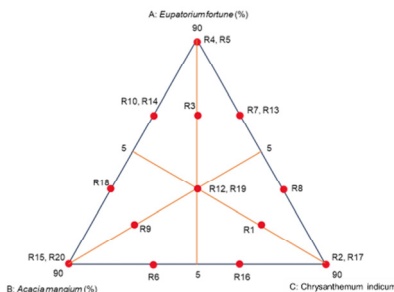


Figure 2.1. SLD design model

Method for evaluating the growth inhibitory activity against *Microcystis aeruginosa*

M. aeruginosa is a unicellular cyanobacterium; therefore, its cell density can be directly counted using a Sedgewick-Rafter counting chamber (20 mm × 50 mm × 1 mm). The number of cells per milliliter was counted under an Olympus-BX51 optical microscope (Japan). The cell density was calculated using the following formula:

$$N_0(\text{mL}^{-1}) = (C \times 1000) / (L \cdot D \cdot W \cdot S)$$

- C: number of cells counted
- L: length of the counting chamber

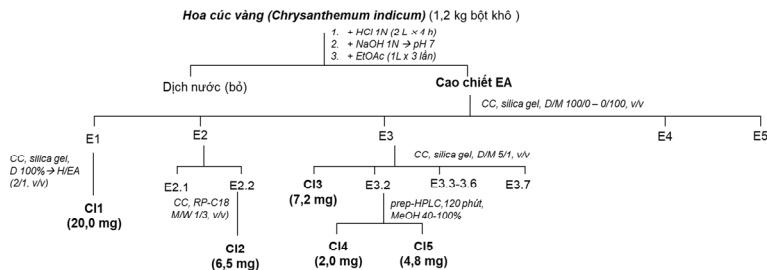
- **D**: depth of the counting chamber
- **W**: width of the counting chamber
- **S**: number of squares counted

Assessment of growth via optical density measurement:

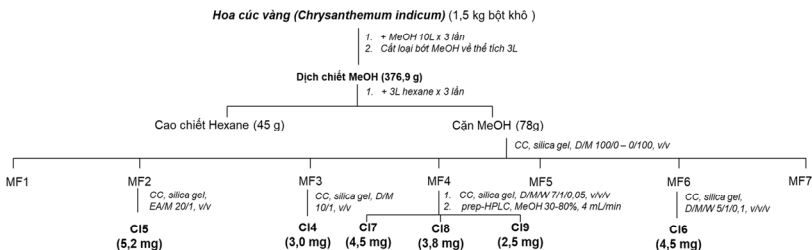
M. aeruginosa was cultured in Sorokin and Krauss nutrient media. Culture samples treated with test compounds at specific concentrations were collected at various time points (T0, T3, T6, and T10). The culture solution was pipetted into wells of a 96-well microplate at each sampling time. The optical density (OD) was then measured at 680 nm using a microplate reader (with three replicates). The inhibition efficiency (IE) was calculated using the following formula:

$$IE (\%) = \frac{OD(V_0) - OD(V_t)}{OD(V_0)} \times 100$$

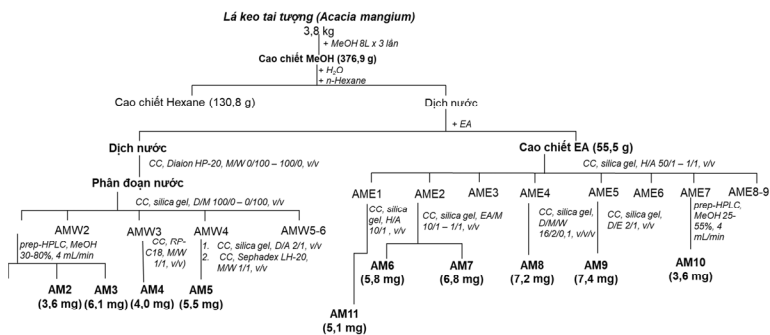
Isolation scheme



*Isolation scheme of compounds from Chrysanthemum indicum
extracted with 1N HCl*



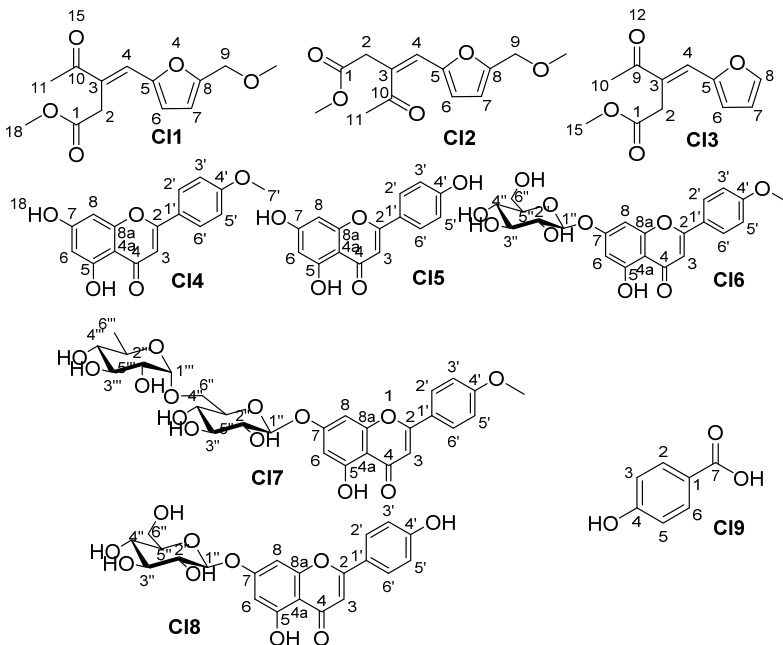
*Isolation scheme of compounds from *Chrysanthemum indicum* extracted with methanol (MeOH)*



*Isolation scheme of compounds from *Acacia mangium* leaves*

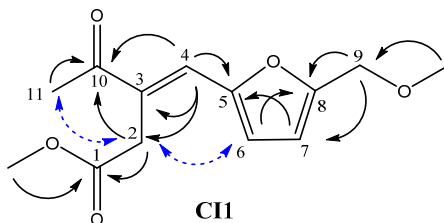
CHAPTER III. RESULTS AND DISCUSSION

3.1. Structural elucidation of compounds from *Chrysanthemum indicum*



Compounds isolated from *Chrysanthemum indicum*

3.1.1. *CI1*



Chemical structure, key HMBC (→) and NOESY (↔) correlations of compound **CI1**

Compound CI1 was determined to have the molecular formula $C_{11}H_{12}O_4$, based on the pseudomolecular ion peak observed at m/z 253.1071 $[M+H]^+$ in the high-resolution mass spectrum. The 1H NMR spectrum of CI1 revealed the presence of three olefinic protons at δ 7.35 (1H, s, H-4), 6.69 (1H, d, $J = 3.5$ Hz, H-6), and 6.45 (1H, d, $J = 3.5$ Hz, H-7). A methylene group directly attached to an oxygen atom appeared at δ 4.18 (2H, s, H-9), and another methylene proton signal was observed at 3.80 (2H, s, H-2). Additionally, signals for methoxy groups were found at δ 3.39 (3H, br s, 9-OCH₃) and 3.67 (3H, br s, COOCH₃), while a methyl signal appeared at 2.44 (3H, br s, H-11).

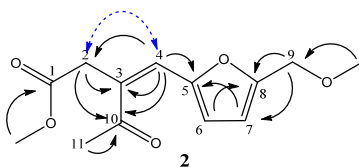
The ^{13}C NMR and HSQC spectra of CI1 showed 13 carbon signals, including three methyl carbons at δ 25.2 (C-11), 58.2 (OCH₃), and 51.9 (COOCH₃); three methine carbons at δ 128.4 (C-4), 117.6 (C-6), and 111.7 (C-7); and other carbon signals at δ 171.5 (C-1), 32.0 (C-2), 130.8 (C-3), 150.7 (C-5), 155.1 (C-8), 66.4 (C-9), and 197.9 (C-10).

The small coupling constant between H-6 and H-7 ($J = 3.5$ Hz), along with the HMBC correlations from H-7 to C-5 and C-8, indicate a 1,4-substituted furan ring. HMBC correlations were observed from H-2 to C-1, C-3, C-4, and C-10; from H-4 to C-2, C-3, C-5, C-6, and C-10; and from H-9 to C-7 and C-8. These spectroscopic data are highly consistent with those of the known compound *(E)*-3-[5-(hydroxymethyl)furan-2-yl]methylene-4-oxopentanoic acid [85], except for the additional presence of two oxymethyl groups.

To determine the geometry of the C-3=C-4 double bond, the NOESY spectrum of CI1 was closely examined. A correlation was observed between H-2 and H-6, but not between H-2 and H-4, indicating an *E* configuration for the double bond.

Therefore, compound CI1 was identified as **(E)**-3-((5-(methoxymethyl)furan-2-yl)methylene)-4-oxopentanoate, a new natural product reported for the first time.

3.1.2. CI2



Chemical structure, key HMBC ($\rightarrow$) and NOESY ($\leftrightarrow$) correlations of compound CI2

Compound CI2 was obtained as a white solid. The ^1H NMR spectrum of CI2 displayed three olefinic proton signals at δ 6.46 (1H, br s, H-4), 6.53 (1H, d, $J = 3.5$ Hz, H-6), and 6.36 (1H, d, $J = 3.5$ Hz, H-7). A methylene group directly bonded to an oxygen atom appeared at δ 4.36 (2H, s, H-9), along with methoxy group signals at δ 3.35 (3H, br s, 9-OCH $_3$) and 3.69 (3H, br s, COOCH $_3$), and a methyl group signal at δ 2.44 (3H, br s, H-11). Two methylene groups were observed at δ 4.36 (2H, s, H-9) and 3.80 (2H, s, H-2).

The ^{13}C NMR and HSQC spectra of CI2 showed 13 carbon signals, including three methyl carbons at δ 29.9 (C-11), 58.0 (OCH $_3$), and 52.1 (COOCH $_3$); three methine carbons at δ 122.0 (C-4), 113.7 (C-6), and 111.3 (C-7); and other carbon signals at δ 171.1 (C-1), 41.0 (C-2), 133.0 (C-3), 149.8 (C-5), 153.4 (C-8), 66.3 (C-9), and 204.3 (C-10).

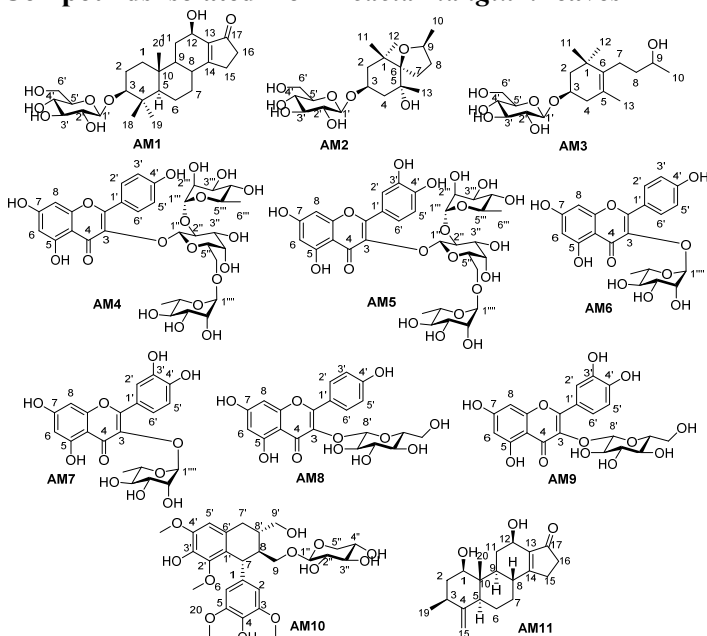
The NMR data of CI2 were nearly identical to those of CI1, except for significant differences in the chemical shifts at C-2, C-3, C-4, and C-10 (see Table 3.1). A notable downfield shift at C-2 in CI2 compared to CI1 suggests a difference in the configuration of the double bond. The NOESY spectrum showed a clear NOE interaction between H-2 and H-4, supporting a *Z* configuration.

Therefore, compound CI2 was identified as **(*Z*)-3-((5-(methoxymethyl)furan-2-yl)methylene)-4-oxopentanoate**, a new natural compound.

*NMR data of **CI1** and **CI2***

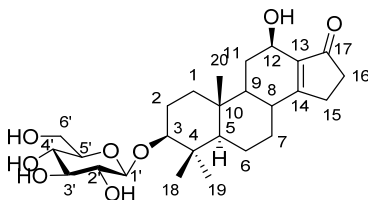
	CI1		CI2	
No	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	-	171.5	-	171.1
2	3.80 (2H; s)	32.0	3.43 (2H; d; 1.0)	41.0
3	-	130.7	-	133.0
4	7.35 (1H; s)	128.4	6.46 (1H; br s)	122.0
5	-	150.6	-	149.8
6	6.69; 1H; d; 3.5	117.6	6.53 (1H; d; 3.5)	113.7
7	6.45; 1H; d; 3.5	111.7	6.36 (1H; d; 3.5)	111.3
8	-	155.0	-	153.4
9	4.42 (2H; s)	66.4	4.36 (2H; s)	66.3
10	-	197.9	-	204.3
11	2.44 (3H; br s)	25.2	2.37 (3H; br s)	29.9
9-OCH ₃	3.38(3H; br s)	58.2	3.35 (3H; br s)	58.0
COOCH ₃	3.67 (3H; br s)	51.9	3.69 (3H; br s)	52.1

3.2. Compounds isolated from *Acacia mangium* leaves



Isolated compounds from *Acacia mangium*

3.2.1. *AM1 (new compound)*



Structure of the new compound acacienoside A

Compound AM1 was isolated as a white powder with a specific optical rotation of $[\alpha]_D^{25} 12.6$ ($c = 0.1$, MeOH). Its molecular formula was determined to be $C_{28}H_{34}O_{10}$, based on the pseudomolecular ion peak at m/z 515.2417 $[M+Cl]^-$ in the HR-ESI-MS spectrum (calculated value: 515.2411 for $C_{28}H_{34}O_{10}Cl$).

The ^1H NMR spectrum exhibited three singlet signals for tertiary methyl groups at δ 0.91 (3H, s, H-19), 1.08 (3H, s, H-18), and 0.97 (3H, s, H-20). Three methine protons appeared at δ 0.95 (1H, m, H-5), 2.45 (1H, m, H-8), and 1.09 (1H, m, H-9). Seven methylene proton signals were observed in the range δ 1.14–2.63 ppm. Additionally, signals for oxygenated methine protons were detected at δ 3.22 (1H, dd, $J = 1.2, 7.6$ Hz, H-3) and δ 4.50 (1H, br m, H-12), together with a series of signals from δ 3.22 to 3.87 ppm and a distinct anomeric proton at δ 4.34 (1H, d, $J = 7.8$ Hz), indicating the presence of a sugar moiety.

The ^{13}C NMR and HSQC spectra confirmed 26 carbon signals, including a ketone carbon at δ 211.3 (C-17), and two oxygenated carbons at δ 90.4 (C-3) and 66.4 (C-12). Two quaternary sp^2 carbons were observed at δ 140.3 (C-13) and 182.3 (C-14). Among them, 20 carbons were assigned to the terpenoid backbone, and 6 to the sugar moiety. These spectroscopic data are comparable to those of acacienone, a labdane-type diterpenoid previously isolated from *Acacia mangium*, with the notable differences being the presence of an additional sugar unit and the hydroxyl group positioned at C-3.

Detailed analysis of 2D NMR spectra (COSY, HMQC, HMBC) established the carbon–proton connectivity. The COSY spectrum indicated partial structures for rings A (H-1/H-2/H-3) and B/C (H-5/H-6/H-7/H-8/H-9, and H-9/H-11/H-12). HMBC correlations from the methyl proton H-20 (δ 0.97) to C-1 (δ 38.1), C-5 (δ 55.6), and C-9 (δ 53.1), along with correlations from H-1, H-6, and H-9 to the quaternary carbon C-10, confirmed the cyclohexane rings A and B joined at C-10. Further HMBC correlations from methyl protons H-18 (δ 1.08) and H-19 (δ 0.91) to C-4 (δ 40.5), C-5, and C-3 (δ 90.4) confirmed two methyl groups attached at C-4. HMBC correlations from the oxygenated methine proton at δ 4.50 to the quaternary sp^2 carbon C-13 (δ 140.3), C-14 (δ 182.3), and ketone carbon C-17 (δ 211.3) confirmed the hydroxyl group at C-12.

Additional HMBC correlations from H-3 (δ 3.22) to C-4 and C-5 further confirmed the hydroxyl group at C-3.

HMBC correlations from H-15 (δ 2.52, 2.63) and H-16 (δ 2.40) to C-17 and the sp^2 carbons C-13 and C-14 suggested the presence of a cyclopentenone ring (ring D), forming an indanone system fused with ring C. The glycosidic linkage at C-3 was confirmed by HMBC correlation from the anomeric proton (δ 4.34) to C-3 (δ 90.4). The coupling constants, chemical shifts, and signal multiplicities indicated that the sugar moiety was β -glucose.

The relative stereochemistry was established by NOESY correlations, showing that protons H-3, H-5, H-9, and H-19 were α -oriented, whereas H-8 and methyl H-20 were β -oriented. A key NOESY correlation between H-7 and H-15 supported the placement of the carbonyl group at C-17 within the cyclopentenone (ring D).

Based on comprehensive spectral analysis, the structure of compound AM1 was determined to be a new labdane-type diterpenoid, and was named **acacienoside A**.

3.3. *M. aeruginosa* inhibitory effects of the isolated compounds

The inhibitory activities of the isolated compounds against *Microcystis aeruginosa* varied significantly. Compounds CI1–CI9 isolated from *Chrysanthemum indicum* and AM4–AM9 from *Acacia mangium* exhibited a wide range of IC_{50} values, from 51.7 to over 100 μ M. Among them, flavonoid aglycones such as acacetin (CI4, $IC_{50} = 51.7 \pm 3.3 \mu$ M) and apigenin (CI5, $IC_{50} = 55.4 \pm 4.5 \mu$ M) demonstrated the strongest inhibitory effects, followed by glycosylated flavonoids including acacetin 7-O- β -D-glucopyranoside (CI6, $IC_{50} = 76.8 \pm 8.6 \mu$ M), acacetin 7-O-rutinoside (CI7, $IC_{50} = 81.3 \pm 7.4 \mu$ M), and apigenin 7-O- β -D-glucopyranoside (CI8, $IC_{50} = 85.5 \pm 6.5 \mu$ M).

Furan derivatives such as compound CI1 exhibited moderate activity ($IC_{50} = 85.0 \pm 7.2 \mu$ M), while its related isomers CI2 and CI3 showed IC_{50} values exceeding 100 μ M, indicating significantly weaker activity. Additionally, p-hydroxybenzoic acid (CI9) demonstrated notable inhibitory effect ($IC_{50} = 62.6 \pm 5.1 \mu$ M).

Glycosylated flavonoid compounds isolated from *Acacia mangium* (AM4–AM7) generally exhibited weak activity with IC₅₀ values above 100 μ M, except for astragalin (AM8, IC₅₀ = 88.7 ± 7.2 μ M) and isoquercetin (AM9, IC₅₀ = 85.9 ± 7.8 μ M), which showed moderate inhibitory activity.

Inhibitory activity against M. aeruginosa of selected isolated compounds

Compound	IC ₅₀ (μ M)
(<i>E</i>)-3-((5-(methoxymethyl)furan-2-yl)methylene)-4-oxopentanoate (CI1)	85.0 ± 7.2
Methyl (<i>Z</i>)-3-((5-(methoxymethyl)furan-2-yl)methylene)-4-oxopentanoate (CI2)	> 100
Methyl (<i>E</i>)-3-(furan-2-ylmethylene)-4-oxopentanoate (CI3)	> 100
Acacetin (CI4)	51.7 ± 3.3
Apigenin (CI5)	55.4 ± 4.5
Acacetin 7- <i>O</i> - β -D-glucopyranoside (CI6)	76.8 ± 8.6
Acacetin 7- <i>O</i> -rutinoside (CI7)	81.3 ± 7.4
Apigenin 7- <i>O</i> - β -D-glucopyranoside (CI8)	85.5 ± 6.5
<i>p</i> -Hydroxybenzoic acid (CI9)	62.6 ± 5.1
Kaempferol-3- <i>O</i> - α -L-rhamnopyranosyl-(1-6)- <i>O</i> -[α -L-rhamnopyranosyl(1-2)]- <i>O</i> - β -D-galactopyranoside (AM4)	>100
Quercetin-3- <i>O</i> - α -L-rhamnopyranosyl-(1-6)- <i>O</i> -[α -L-rhamnopyranosyl(1-2)]- <i>O</i> - β -D-galactopyranoside (AM5)	>100
Afzelin (AM6)	>100
Quercitrin (AM7)	>100
Astragalin (AM8)	88.7 ± 7.2
Isoquercetin (AM9)	85.9 ± 7.8
CuSO ₄	41.8 ± 3.9

3.4. Optimize the mixing ratio of the extracts for the inhibition of *Microcystis aeruginosa*

3.4.1. Bioactivities evaluation

3.4. Determination of the optimal blending ratio for extract mixtures inhibiting *Microcystis aeruginosa*

3.4.1. Evaluation of the activity of individual extracts and selected extract mixtures

Data on the inhibitory effect of individual and combined (2–3 component) plant extracts at a concentration of 200 µg/mL against *Microcystis aeruginosa* are summarized in the table below.

Inhibitory activity of crude extracts and extract mixtures against M. aeruginosa

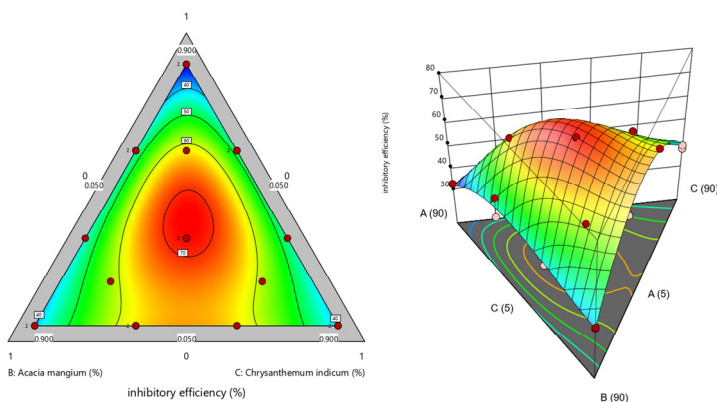
	<i>Eupatorium fortune</i> (Ef) (%)	(Am) (%)	<i>Chrysanthemum indicum</i> (Ci) (%)	% I
MC_Ef	100.00	0.00	0.00	29.31 ± 1.24
MC_Am	0.00	100.00	0.00	26.58 ± 1.57
MC_Ci	0.00	0.00	100.00	30.26 ± 3.02
MC_EfAm	50.00	50.00	0.00	38.73 ± 2.59
MC_EfCi	50.00	0.00	50.00	37.67 ± 1.97
MC_AmCi	0.00	50.00	50.00	39.25 ± 2.11
MC_EAC	33.33	33.33	33.33	71.63 ± 6.58
Control	CuSO ₄			52.69 ± 3.36

The results demonstrated that all three individual extracts exhibited inhibitory activity, with inhibition efficiencies ranging from 26.5% to 30.2%. Among them, the extract from *Chrysanthemum indicum* (Ci) showed the highest inhibition (30.2%), slightly outperforming *Eupatorium fortunei* (Ef, 29.3%) and *Acacia mangium* (Am, 26.5%).

When two extracts were combined, the inhibitory activity was notably enhanced. The Am + Ci mixture achieved an inhibition of 39.2%, higher than that of Ef + Am (38.7%) and Ef + Ci (37.6%), indicating synergistic effects between different plant sources.

Remarkably, the ternary mixture of equal parts (Ef:Am:Ci = 1:1:1), designated as MC_EAC, exhibited the highest inhibitory effect, reaching 71.6%. This value is nearly twice that of any individual extract and significantly higher than those of binary mixtures, suggesting pronounced synergism or additive effects when all three extracts are combined. Compared to the positive control (CuSO₄, 52.6% inhibition), MC_EAC showed substantially superior efficacy. This finding highlights that the combination of all three extracts provides much greater inhibition than any single extract alone.

3.4.2. Optimal blending ratio of extract mixture for inhibiting *Microcystis aeruginosa*



Response surface of extract ratios and inhibition efficiency (%) against M. aeruginosa

Based on the selected model, optimal extract ratios were calculated and proposed as shown below.

Optimal blending ratios suggested by RSM for extract mixtures

	% <i>E. fortunei</i>	% <i>A. mangium</i>	% <i>C. indicum</i>	%IE predict
1	36.39	30.45	33.16	71.80
2	41.68	27.80	30.52	71.77
3	39.77	31.85	28.38	71.70
4	39.92	30.11	29.97	71.90
5	35.17	31.89	32.93	71.71

As shown in the table, the proportion of *Eupatorium fortunei* extract ranged from 35.17% to 41.68%, *Acacia mangium* from 27.80% to 31.89%, and *Chrysanthemum indicum* from 28.38% to 33.16%. These variations, ranging from 4% to 6%, indicate a stable optimal region where small changes in blending ratios do not significantly affect biological performance.

In terms of predicted inhibition efficiency, all formulations yielded very high and comparable values, ranging from 71.70% to 71.90%, with a minimal difference of only 0.20%. This suggests that the predictive model describes a broad optimal region in which multiple extract ratio combinations can achieve similarly high levels of inhibitory activity.

Based on these data, we propose a blending formulation consisting of 40% *E. fortunei*, 30% *A. mangium*, and 30% *C.*

indicum. This formulation lies well within the defined optimal range and is close to the combinations with the highest predicted efficacy. Moreover, the simplicity and balance of this ratio offer practical advantages for preparation, scaling, and application, while still maintaining optimal inhibition of *M. aeruginosa*.

Validation of this proposed formulation showed an actual inhibition efficiency (%IE) of $72.95 \pm 4.74\%$, which is higher than the theoretical value predicted by the model. This confirms that the mixture containing 40% *E. fortunei*, 30% *A. mangium*, and 30% *C. indicum* provides optimal effectiveness for inhibiting the growth of *Microcystis aeruginosa*

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

This dissertation has achieved significant outcomes in the study of chemical constituents and the evaluation of inhibitory effects against *Microcystis aeruginosa* from *Chrysanthemum indicum* flowers and *Acacia mangium* leaves. Specifically:

1. Nine compounds were isolated and structurally elucidated from *C. indicum*, including three furan derivatives (two new compounds and one reported with complete NMR data for the first time), flavonoid derivatives, and a phenolic acid. Among them, flavonoid aglycones such as **acacetin** and **apigenin** exhibited the strongest inhibitory activity against *M. aeruginosa*.
2. From *A. mangium*, eleven compounds were isolated and identified, comprising two new terpenoid glycosides, six flavonoid glycosides, one lignan glycoside, and two known terpenoids. Notably, **astragalinalin** and **isoquercetin** demonstrated moderate inhibitory activity against the cyanobacterium.
3. Crude extracts from *C. indicum* and *A. mangium*, as well as several isolated compounds (e.g., acacetin and apigenin), exhibited inhibitory effects on *M. aeruginosa*.
4. The study on synergistic blending of extracts from *C. indicum*, *A. mangium*, and *Eupatorium fortunei* identified an optimal ratio of **40% *E. fortunei*, 30% *A. mangium*, and 30% *C. indicum***. This combination exhibited significantly enhanced inhibitory activity ($72.95 \pm 4.74\%$), outperforming individual extracts and binary mixtures

RECOMMENDATIONS

1. Conduct pilot-scale field trials to evaluate the efficacy and feasibility of using these plant extracts for cyanobacterial control in reservoir ecosystems.
2. Further investigate additional biological activities such as antioxidant, anti-inflammatory, and toxicity profiles in animal models to ensure safety and explore broader applications of the compounds and extracts.

NOVEL CONTRIBUTIONS OF THE DISSERTATION

1. For the first time, several new furan compounds were isolated and structurally elucidated from *Chrysanthemum indicum*.
2. This study provides the first systematic evaluation and comparison of the inhibitory activity against *Microcystis aeruginosa* of flavonoid compounds isolated from *Chrysanthemum indicum* and *Acacia mangium*.
3. A predictive model was successfully established to optimize the blending ratios of plant extracts from *Chrysanthemum indicum*, *Acacia mangium*, and *Eupatorium fortunei* for the development of a highly effective biopreparation for cyanobacterial control, offering promising practical applications in aquatic environmental management.

LIST OF PUBLICATIONS RELATED TO THE THESIS

- [1] Nguyen Van Phuong, Le Thi Phuong Quynh, Le Nhu Da, Nguyen Thi Hong Anh, Do Hoang Giang, Nguyen Thi Luyen, Truong Ngoc Minh, Duong Thi Thuy, Nguyen Tien Dat (2025) Antibacterial Furan Derivatives from the Flowers of *Chrysanthemum indicum* L. *BioResources* 20(1), 1188-1199.
- [2] Nguyễn Văn Phương, Nguyễn Thu Uyên, Đỗ Hoàng Giang, Nguyễn Thị Thu Minh, Hoàng Thùy Dương, Bùi Thị Nhật Lệ, Lưu Hải Nhi, Lê Thị Phương Quỳnh, Nguyễn Tiến Đạt (2023) Một số hợp chất Flavonoid glycoside phân lập từ lá cây keo tai tượng (*Acacia magium*). *Tạp chí phân tích Hoá, Lý và Sinh học* 29(4), 45-51