Hindawi
Journal of Chemistry
Volume 2023, Article ID 7977620, 9 pages
https://doi.org/10.1155/2023/7977620

Research Article

Antiproliferative, Anti-Inflammatory Activities, and Molecular Docking Studies of Secondary Metabolites from *Macrosolen tricolor*

Van Son Dang,[1,2] Kieu Hung Le,[3] Thi Hong Tuoi Do,[4] Le Thanh Tuyen Nguyen,[5] Thi Phuong Lan Vu,[6] Thi Kim Chi Huynh,[1,7] Thi Kim Dung Hoang,[1,7] Quan Hien Nguyen,[8] Trong Nghia Ngo,[9] Diep Xuan Ky Nguyen,[7] Nhat Minh Phan,[1,7] Trong Dat Bui,[7] Dinh Tri Mai,[1,7] and Tan Phat Nguyen [1,7]

[1]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[2]*Institute of Tropical Biology, Vietnam Academy of Science and Technology, 85 Tran Quoc Toan, Ho Chi Minh City, Vietnam*
[3]*Le Quy Don High School for the Gifted, Vung Tau, Ba Ria-Vung Tau Province, Vietnam*
[4]*Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam*
[5]*Saigon Pharmaceutical Sciences & Technologies Center, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam*
[6]*Ton Duc Thang University, Ho Chi Minh City, Vietnam*
[7]*Institute of Chemical Technology, Vietnam Academy of Science and Technology, 01ATL29 Thanh Loc, District 12, Ho Chi Minh City, Vietnam*
[8]*Institute of Applied Mechanics and Informatics, Vietnam Academy of Science and Technology, 291 Dien Bien Phu, District 03, Ho Chi Minh City, Vietnam*
[9]*Kien Giang University, Chau Thanh, Kien Giang Province, Vietnam*

Correspondence should be addressed to Tan Phat Nguyen; phat_nguyentan88@yahoo.com

Received 12 September 2022; Revised 13 December 2022; Accepted 21 December 2022; Published 2 January 2023

Academic Editor: Mahmood Ahmed

In Vietnam, *Macrosolen tricolor* is used for the treatment of bloating, broken bones, cough, diarrhea, diuretic, rheumatism, and laxative effects. The study aimed to identify the *in vitro* antiproliferation and anti-inflammation of all fractions and purified compounds from the *M. tricolor* whole plants, as well as the *in silico* molecular docking of the potentially cytotoxic compounds. As the results, fractions (**MTH.I**, **MTH.II**, **MTE.I**, and **MTE.II**) strongly demonstrated antiproliferative properties against three tested cells, MDA-MB-231, RD, and HepG2 (IC_{50} values ranged from 4.00 ± 0.20 to $70.60 \pm 1.44\,\mu$g/mL), as well as anti-inflammatory effects (IC_{50} values ranged from 4.45 ± 0.08 to $23.00 \pm 1.18\,\mu$g/mL), whereas other fractions meaningfully evidenced selective cytotoxicity and/or anti-inflammation. Therefore, the phytochemical compositions of the active fractions were illuminated, leading to the characterization of eighteen compounds. Compounds (**3**–**5**) revealed the most cytotoxic effects towards all examined cells (IC_{50} values ranged from 6.88 ± 0.12 to $71.64 \pm 1.17\,\mu$M) and the strongest anti-inflammatory properties (IC_{50} values of 16.30 ± 0.92, 7.31 ± 0.55, and $9.23 \pm 0.60\,\mu$M, respectively). Compound **11** showed potential cytotoxicity against MDA-MB-231, RD, and HepG2 cells (IC_{50} values of 24.42 ± 0.28, 20.60 ± 0.25, and $3.20 \pm 0.02\,\mu$M, respectively). Furthermore, compounds (**4**, **5**, and **11**) interacted with the active site of the apoptosis regulator Bcl-2 protein (PDB ID: 2O2F), were comparable to PAC, and were compatible with their anticancer activity. This project suggests that *M. tricolor* is a good source of natural antiproliferative and anti-inflammatory agents and contributes to understanding the biological activities of *Macrosolen* species in traditional Vietnamese medicine.

1. Introduction

Inflammation, characterized as the first protective response of the immune system, is a common and highly dynamic process in the body. The extent of the inflammatory response is seriously important. Chronic inflammation results from an insufficient inflammatory response, which is implicated in the pathophysiology of cancers [1, 2]. In this process, macrophages express the vital administration of many immune-pathological phenomena, including the overproduction of major inflammatory mediators such as NO [2, 3]. Therefore, the investigation of bioactive components from herbal medicines possessing antiproliferation [4] and anti-inflammation properties against NO overproduction [5, 6] is very essential and of most concern today.

Macrosolen tricolor, belonging to the Loranthaceae family, is a hemiparasitic shrub, ca. 30–50 cm. The branches are grayish. The leaves are blade obovate to narrowly obovate, 3.5–5.5 × 1.3–2 cm, have 2 or 3 pairs of lateral veins, and the petiole is 2–3 mm long. The flowers consist of solitary or paired inflorescences, a peduncle of ca. 1 mm, and a mature bud of 2.5–3.5 cm. The corolla is red with a green band at the top of the tube, slightly curved, tube inflated, lobes greenish, lanceolate, 6–9 mm, reflexed. Berries are dark purple, globose, ca. 7 mm, and smooth. The plant is used in traditional Vietnamese medicine for treatments such as bloating, broken bones, cough, diarrhea, diuretic, rheumatism, and laxative effects [7, 8].

In vivo pharmacological studies on rats demonstrated that the liquid of the *M. tricolor* whole plants provided hepatoprotection against paracetamol-induced hepatotoxicity, as evidenced by decreased liver weight and aspartate aminotransferase (ALT) and alanine aminotransferase (AST) concentrations in the blood (at the doses of 30 g/kg and 60 g/kg, which were compared to silymarin at a dose of 70 mg/kg) [9, 10]. *M. tricolor* whole plant liquid was also found to have anti-inflammatory effects on carrageenan-induced paw oedema and carrageenan plus formaldehyde-induced abdominal writhing (at a dose of 40 g/kg, compared to the aspirin drug's dose of 150 mg/kg) [11, 12].

Lupeol, 3β-nonadecanoyllup-20(29)-ene-7β-ol, 3β-nonadecanoyllup-20(29)-ene-7β,15α-diol, quercetin 3-rhamnoside, and methyl brevifolin carboxylate were discovered in the *M. tricolor* whole plants [3–5]. In our earlier papers, one diarylpropanoid, three diarylheptanoids, three phenolics, three flavonoids, and two steroids were characterized from this species [7, 8].

There is no report on the *in vitro* antiproliferative and anti-inflammatory properties of this species. Continuing our exciting studies on the cytotoxic constituents of the Vietnam plants [13–15] and the *Macrosolen* genus [16–19], this study disclosed the *in vitro* cytotoxic efficacy on MDA-MB-231, RD, HepG2, and RAW264.7 cells and anti-inflammatory evaluation against NO production in LPS-induced inflammation of fractions and separated compounds using MTT assay, as well as the *in silico* molecular docking studies on the Bcl-2-regulated apoptosis protein for the most cytotoxic isolates.

2. Materials and Methods

2.1. Plant Material. The *Macrosolen tricolor* whole plants, certificated by Dr. Dang Van-Son, Institute of Tropical Biology, were collected in Ba Ria-Vung Tau Province (December 2018) and further deposited in the Bioactive Compounds Laboratory, Institute of Chemical Technology (voucher herbarium specimen No. VH/PHAT-MT1218).

2.2. Extraction. The whole plants of *M. tricolor* were washed, dried, and powdered (50 mesh). The ground powder sample (4.3 kg) was macerated using 96% ethanol at room temperature to deliver the crude extract (MTEt). The MTEt extract (1.1 kg) was applied to two liquid-phase separations and consecutively fractionated with *n*-hexane (*n*-H) and ethyl acetate, respectively**,** to obtain MTH (100 g), MTE (106 g) extracts, and water layer (1000 g).

The MTH extract was separated on a silica gel column and eluted with *n*-H/EtOAc (100/0–0/100, v/v) to yield five fractions: **MTH.I** (10.0 g), **MTH.II** (50.0 g), **MTH.III** (5.0 g), **MTH.IV** (15.0 g), and **MTH.V** (12.0 g). Similarly, the MTE extract was segregated on normal-phase column chromatography (CC) eluting with *n*-H/EtOAc/MeOH (25/75/0–0/90/10, v/v/v) to get five fractions: **MTE.I** (18.0 g), **MTE.II** (5.2 g), **MTE.III** (18.2 g), **MTE.IV** (30.0 g), and **MTE.V** (9.0 g). Those were stored at 4°C for further investigation.

2.3. Chemicals and Reagents. Penicillin (PEN), *L*-glutamine (GLU), phosphate buffer, streptomycin (STR), carbon tetrachloride (CCl_4), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and PAC were purchased from Sigma-Aldrich. Fetal calf serum (FCS), Eagle's Minimum Essential Medium (EMEM), and trypsin-Ethylene Diamine Tetracetic Acid (EDTA) were ordered from Gibco. Isopropanol, ethanol, and dimethyl sulfoxide were delivered from Merck. All chemicals met regulatory requirements.

2.4. Cell Culture. All tested cell lines (RAW264.7, MDA-MB-231, RD, and HepG2 cells) required from the ATCC organization in Virginia were seeded and cultured in EMEM including 2 mM GLU, 10% FCS (v/v), 100 IU/mL PEN, and 100 μg/mL STR (in 5% CO_2 at 37°C).

2.5. MTT Assay. The *in vitro* cytotoxic effects were observed against four tumor cells: MDA-MB-231, RD, HepG2, and RAW264.7 of all MTH, MTE fractions, and pure compounds were exposed by the MTT test, as described in our previous papers [15, 16].

Cancer cells (MDA-MB-231, RD, and HepG2) were harvested and seeded in 96-wells at 4.0×10^4 cells/cm^2 in EMEM or DMEM medium supplemented with 2 mM GLU, 10% FCS, 100 μg/mL STR, and 100 IU/mL PEN. Cells were treated with tested fractions, purified compounds at different concentrations, PAC, and blank control (DMSO), after 24 h of incubation (in 5% CO_2 at 37°C). Cell media were removed from the plate, the cells were washed using 200 μL buffered

saline (PBS), and MTT solution was added to each well, after 72 h of incubation (in 5% CO_2 at 37°C). Succinate dehydrogenase (SDH) activity was detected after 4 h of incubation at 37°C, which is converted into formazan dissolved in isopropanol by agitation for 10 min at room temperature. The absorbance was measured at 570 nm. The IC_{50} values were calculated by regressing the concentrations of the tested fractions (at 12.5, 25.0, 50.0, and 100.0 μg/mL) and the cell inhibition percentages. The positive drug, PAC was purposed.

2.6. NO Assay. The *in vitro*anti-inflammation of LPS-induced inflammatory effect using RAW264.7 cells of all MTH, MTE fractions, and isolated compounds were communicated as in previous papers [7, 20].

RAW264.7 cells derived from mice bone marrow were seeded into 96-well plates. Cells were incubated for 3 hours and then treated with 04 high testers at the different concentrations for 30 minutes. The cells were then treated without or with a 1 μg/mL LPS to induce inflammation, and incubated for 24 hours (in 5% CO_2 at 37°C). After 24 hours, each well received 100 μL of culture medium and 100 μL of Griess reagent. The nitrite concentration was measured in the supernatants of tissue homogenate with 1% bovine serum albumin. Then an equal volume of the sample with Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% N-[1-Napthyl]-ethylenediamine) was mixed. The absorbance was measured (at 450 nm) to determine the amount of NO. The amount of nitrite was obtained by extrapolation from a standard curve with $NaNO_2$ and expressed as μmol/mg tissue. The positive control, QUE, was serviced.

2.7. General Experimental Procedures for Isolation and Structural Elucidation. The high-resolution mass spectroscopy (HRMS) using the electrospray ionization technique (ESI) was measured on a Sciex spectrometer (Modem X500R-QTOF). The 1D and 2D NMR spectra were recorded on a Bruker Avance spectrometer (Modem AM500, 500 MHz). CC was carried out using normal-phase silica gel (230–400 mesh) and reversed-phase silica gel RP-C_{18} (Merck, Germany). Thin-layer chromatography (TLC) was applied to silica gel 60 F_{254} plates (Merck, Germany). The compounds were sprayed with the H_2SO_4 10%/ethanol reagent and visualized by heating (3–5 min).

2.8. Isolation. The fraction **MTH.I** (10.0 g) was subjected to silica gel CC eluted with the solvent systems of *n*-H/EtOAc (5/95–30/70, v/v) to provide four subfractions (MTH.I.1–MTH.I.4). Subfraction MTH.I.2 (4.5 g) was isolated by normal-phase CC using $CHCl_3$/MeOH (99/1, v/v) to yield **1** (350 mg).

The fraction **MTH.II** (50.0 g) was eluted over a silica gel column with *n*-H/EtOAc systems (20/80–45/55, v/v) to get six subfractions (MTH.II.1–MTH.II.6). Subfraction MTH.II.1 (6.5 g) was chromatographed on normal-phase silica gel using $CHCl_3$/MeOH (99/1, v/v) and on reversed-phase silica gel eluting MeOH/H_2O (20/80, v/v) to afford **1** (160 mg), **2** (8 mg), **3** (5 mg), **4** (4.5 mg), and **5** (7 mg).

The fraction **MTH.III** (5.0 g) was fractioned on a normal-phase column with an *n*-H/EtOAc gradient (30/70–50/50, v/v) to collect five subfractions (MTH.III.1–MTH.III.5). Subfraction MTH.III.1 (2.4 g) was rechromatographed on CC with $CHCl_3$/MeOH solvents (99/1, v/v) and on RP-C_{18} with MeOH/H_2O (30/70, v/v) to give **3** (4 mg), **4** (4 mg), **5** (4 mg), and **6** (4.5 mg).

The fraction **MTH.IV** (15.0 g) was divided into four subfractions (MTH.IV.1–MTH.IV.4) using *n*-H/EtOAc solvents (40/60–70/30, v/v). Subfraction MTH.IV.2 (6.0 g) was purified with $CHCl_3$/MeOH (98/2, v/v) to deliver **7** (8 mg) and **8** (100 mg).

In the same way, fraction **MTE.I** was subjected to CC using *n*-H/EtOAc mixtures (25/75–0/100, v/v) to furnish compounds **1** (200 mg), **8** (35 mg), and a mixture of **3**–**5** (40 mg). Compounds **9** (6 mg), **10** (4 mg), **11** (40 mg), **12** (7 mg), **13** (3.5 mg), **14** (8 mg), **15** (10 mg), and **16** (4 mg) were supplied from fraction **MTE.II**. Finally, compounds **17** (5 mg) and **18** (40 mg) were donated from fraction **MTE.IV**.

2.9. Molecular Docking Studies

2.9.1. Preparation of Compounds. The 2D and 3D chemical structures of compounds **4**, **5**, **11**, and PAC were constructed using ChemDraw 19.1 and MOE 2015.10 software. The structural compounds were optimized by energy minimization and molecular dynamic functions in Sybyl-X 1.1. In energy minimization, the method of minimizing was a conjugate gradient, and the structures of compounds were optimized until a minimal energy change of 0.001 kcal/mol was reached. Gasteiger-Huckel charges were applied to the structure atoms, and the maximal number of iterations was fixed at 10,000 to be performed during minimization. The simulated annealing method was used in this process, and the compounds were heated at 700°K for 1000 femtoseconds and then cooled to 200°K for the same period to achieve stable states from which their final conformations were obtained. This action was conducted in five cycles to discover the different required structures. Lastly, the energy minimization procedure was conducted one more time, and the minimal energy of the final conformations was explored.

2.9.2. Preparation of Protein. The X-ray crystallographic structure of the antiapoptotic protein Bcl-2 in complex with an acyl-sulfonamide-based ligand was collected from the Protein Data Bank (PDB ID: 2O2F) and performed as the receptor model [21]. The 3D structure of the crystallographic complex was established in AutoDock 4.2.6 software [22] to add hydrogen, protonate, and delete unbound waters. The active site was defined by considering a grid box of appropriate size around the bound co-crystal ligand. The grid box dimensions were as follows: no. of grid points $65 \times 65 \times 65$; center (xyz coordinates) −0.024, 3.142, −0.361; grid point spacing (Å) 0.375. The compounds were docked using AutoDock 4.2.6 software.

Table 1: The cytotoxic activity of all fractions of *M. tricolor*.

No	Samples	IC_{50} (μg/mL)			
		MDA-MB-231	RD	HepG2	RAW264.7
1	**MTH.I**	**27.98 ± 2.47**	**24.64 ± 1.12**	**28.51 ± 0.59**	>100
2	**MTH.II**	**10.24 ± 1.73**	**7.09 ± 0.44**	**34.16 ± 1.15**	>100
3	**MTH.III**	**31.24 ± 3.12**	**42.14 ± 2.74**	>100	>100
4	**MTH.IV**	**57.72 ± 2.17**	**73.94 ± 1.89**	>100	>100
5	**MTH.V**	>100	**31.49 ± 0.31**	>100	>100
6	**MTE.I**	**4.00 ± 0.20**	**5.53 ± 0.51**	**33.00 ± 1.11**	>100
7	**MTE.II**	**27.33 ± 1.79**	**20.70 ± 0.24**	**70.60 ± 1.44**	>100
8	**MTE.III**	>100	**96.03 ± 1.89**	>100	>100
9	**MTE.IV**	>100	>100	>100	>100
10	**MTE.V**	>100	>100	>100	>100
11	PAC	**8.38 ± 0.75** (μM)	**5.73 ± 0.27** (μM)	**16.38 ± 0.50** (μM)	

Bold values should be the IC_{50} values below <100.

2.9.3. Evaluation of Docking Results. The docking experiment was organized using the Lamarckian genetic algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2.5×10^6 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.8. One hundred independent docking runs were detailed for each compound. Conformation clustering was carried out considering a root mean square deviation (RMSD) cut-off of 2.0 Å clustered, and the most possible binding modes were described using the lowest free energy of binding (ΔG) and the lowest inhibition constant (K_i). The most favorable binding conformation was selected and evaluated for molecular interaction with their receptors using LigPlot + version 1.4.5 software [23]. To establish that the binding pose of the docked compound defines a probable and valid perspective conformation, the docking parameters and methods were confirmed by redocking the cocrystal ligand against their specific targets. 3D poses of compounds with the antiapoptotic protein Bcl-2 in the complex were displayed using PyMOL 2.5.

3. Results and Discussion

3.1. Antiproliferative Activity against Tumor Cells. The *in vitro* anticancer effects of all fractions of the MTH and MTE extracts were recognized against four tumor cell lines, MDA-MB-231, RD, HepG2, and RAW264.7, following the MTT assay (Table 1).

As the testification, fractions (**MTH.I**, **MTH.II**, **MTE.I**, and **MTE.II**) evidenced meaningful properties against three tested cells, MDA-MB-231, RD, and HepG2 (IC_{50} values ranged from 4.00 ± 0.20 to 70.60 ± 1.44 μg/mL). Moreover, fractions (**MTH.III** and **MTH.IV**) evidenced selective cytotoxicity against MDA-MB-231 and RD (IC_{50} values ranged from 31.24 ± 3.12 to 73.94 ± 1.89 μg/mL), as well as fractions (**MTH.V** and **MTE III**), which proved moderate activity against RD (IC_{50} values of 31.49 ± 0.31 and 96.03 ± 1.89 μg/mL, respectively). It is the first time that the *in vitro* cytotoxic effects of fractions of *M. tricolor* have been reported. Besides, all fractions did not exhibit cytotoxicity toward RAW264.7.

Therefore, the *in vitro*anti-inflammation of LPS-induced NO production in RAW264.7 cells of all MTH and MTE fractions were further examined.

3.2. Anti-Inflammatory Activity on LPS-Induced NO Production in RAW264.7 Cells. The *in vitro*anti-inflammatory properties of LPS-induced inflammation using RAW264.7 cells of all MTH and MTE fractions were certified (Table 2).

As a result, all MTH and MTE fractions except fraction **MTE.III** expressed a significant anti-inflammatory effect (IC_{50} values ranged from 4.45 ± 0.08 to 85.76 ± 8.07 μg/mL). Particularly, fractions (**MTE.I** and **MTH.II**) revealed more powerful efficacy against NO production (IC_{50} values of 4.45 ± 0.08 and 9.78 ± 0.58 μg/mL, respectively) than QUE (IC_{50} value of 10.46 ± 0.16 μg/mL). *In vivo*anti-inflammation of *M. tricolor* whole plant liquid against carrageenan-induced and carrageenan plus formaldehyde-induced in rats was reported [5, 6]. However, it is the first time that the *in vitro*anti-inflammatory activities of fractions from this species were perceived against NO production in RAW264.7 cells. Thus, the active fractions were further elaborated on in terms of phytochemical composition.

3.3. Phytochemical Ingredients of the Active Fractions. The *in vitro*anti-inflammatory and anticancer fractions from the whole plants of *M. tricolor* were administered in normal-phase and reversed-phase CC to afford eighteen compounds (Figure 1).

The HRMS (ESI technique) and NMR spectra of those compounds were consistent with the data in the published literature for lupeol (**1**) [24]; macrotricolorin A **(2)**; bisdemethoxycurcumin (**3**); desmethoxycurcumin (**4**); curcumin (**5**) [7]; 24-methylenecycloartanol (**6**) [25]; 6′-O-margaroyldaucosterol (**7**); daucosterol (**8**); ursolic acid (**9**) [26]; 1-oxooleanolic acid (**10**) [27]; gallic acid (**11**); bisacurone A (**12**) [28]; a mixture of (1E, 4E)-1,5-*bis*(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (**13a**); and pinoresinol (**13b**) [29]; 3,3′-di-O-methylellagic acid (**14**); 3,3′-di-O-methyl-4-O-β-D-xylopyranosylellagic acid (**15**); kaempferol (**16**); vitexin (**17**); and orientin (**18**) [30].

3.4. Antiproliferation and Anti-Inflammation of Purified Compounds. The antiproliferation against MDA-MB-231, RD, and HepG2 cells, and therefore the anti-inflammation against NO production on RAW264.7 cells of purified compounds (**1**–**11**) are shown (Table 3).

Table 2: The anti-inflammatory activity of all fractions of *M. tricolor*.

No	Samples	IC_{50} (μg/mL)
1	**MTH.I**	**12.64 ± 0.77**
2	**MTH.II**	**9.78 ± 0.58**
3	**MTH.III**	**32.81 ± 3.02**
4	**MTH.IV**	**27.42 ± 2.07**
5	**MTH.V**	**51.12 ± 4.51**
6	**MTE.I**	**4.45 ± 0.08**
7	**MTE.II**	**23.00 ± 1.18**
8	**MTE.III**	>100
9	**MTE.IV**	**85.76 ± 8.07**
10	**MTE.V**	**83.90 ± 8.05**
11	**QUE**	**10.46 ± 0.16**

The bold values should be the IC_{50} values below 100.

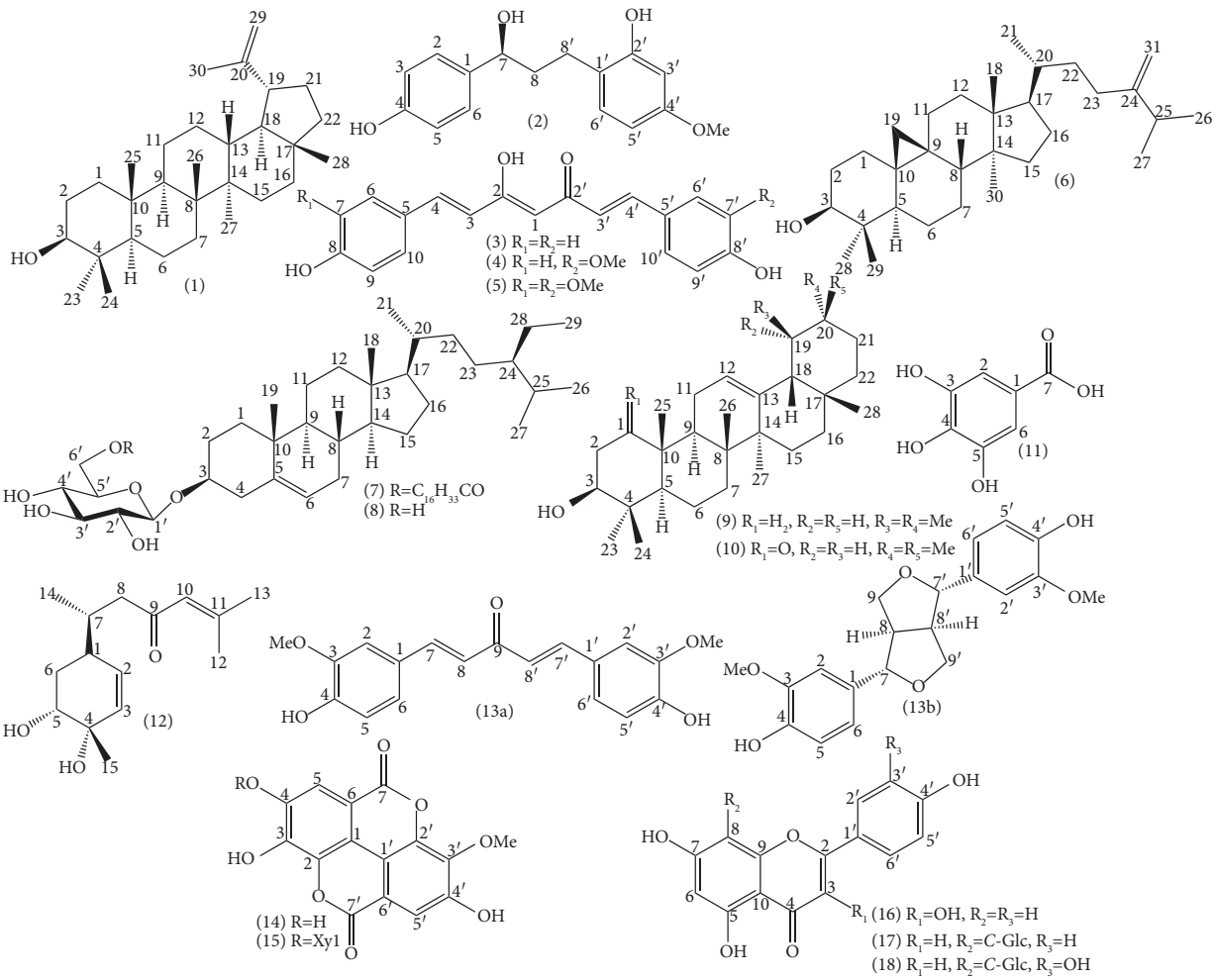


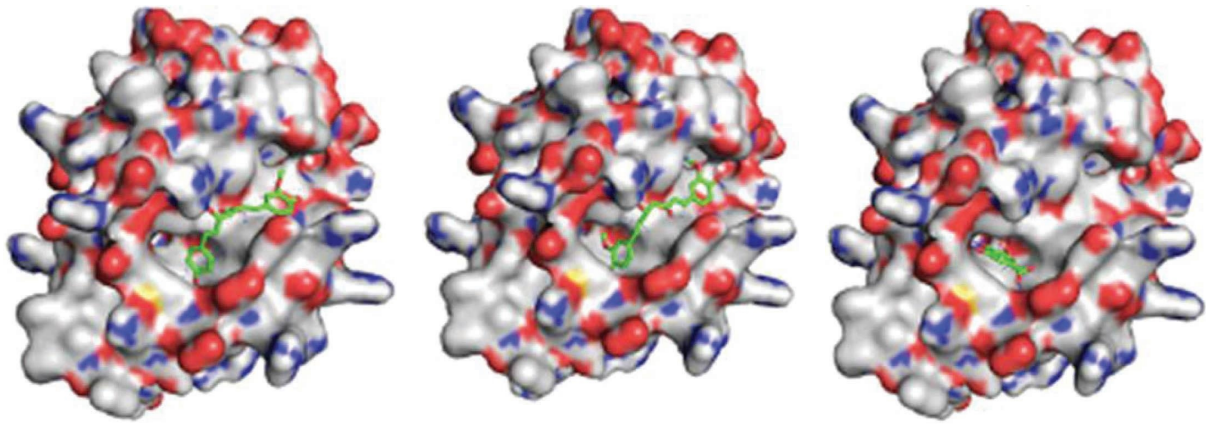
Figure 1: Chemical structures of isolated compounds (**1**–**18**) from *M. tricolor*.

Compounds (**3**–**5**) certificated the strong activity in both antiproliferation against MDA-MB-231, RD, and HepG2 cells (IC_{50} values ranged from 6.88 ± 0.12 to $71.64 \pm 1.17\,\mu$M), and anti-inflammatory activity against NO production on LPS-inducedanti-inflammation using RAW264.7 (IC_{50} values of 16.30 ± 0.92, 7.31 ± 0.55, and $9.23 \pm 0.60\,\mu$M, respectively). Likewise, compound **11** meaningfully showed a cytotoxic effect on all examined cells (IC_{50} values of 24.42 ± 0.28, 20.60 ± 0.25, and $3.20 \pm 0.02\,\mu$M, respectively); however, it didn't display anti-inflammatory activity. Additionally, compound **1** exhibited selective properties against MDA-MB-231 and RD cells (IC_{50} values of 57.78 ± 1.95 and $74.25 \pm 0.46\,\mu$M, respectively), while compounds (**14** and **18**) revealed a moderate effect on RD cells (IC_{50} values of 95.60 ± 1.03 and $65.73 \pm 5.05\,\mu$M, respectively).

Table 3: The antiproliferative and anti-inflammatory activities of compounds (**1**–**11**) from *M. tricolor*.

No	Compounds	IC_{50} (μM)			
		MDA-MB-231	RD	HepG2	NO production
1	**1**	**57.78 ± 1.95**	**74.25 ± 0.46**	>100	—
2	**2**	>100	>100	>100	27.54 ± 1.75*
3	**3**	**59.01 ± 1.24**	**22.79 ± 0.42**	**71.64 ± 1.17**	**16.30 ± 0.92**
4	**4**	**17.28 ± 0.27**	**6.88 ± 0.12**	**28.04 ± 0.41**	**7.31 ± 0.55**
5	**5**	**22.89 ± 0.09**	**9.57 ± 0.18**	**19.92 ± 0.55**	**9.23 ± 0.60**
6	**6**#	>50	>50	>50	>50
7	**11**	**24.42 ± 0.28**	**20.60 ± 0.25**	**3.20 ± 0.02**	>100
8	**14**	>100	**95.60 ± 1.03**	>100	>100
9	**14**	>100	>100	>100	>100
10	**17**	>100	>100	>100	>100
11	**18**	>100	**65.73 ± 5.05**	>100	>100
12	PAC	**8.38 ± 0.75**	**5.73 ± 0.27**	**16.38 ± 0.50**	—
13	QUE				**10.46 ± 0.16**

Bold values should be the IC_{50} values below <100.



(a) (b) (c)

Figure 2: 3D interaction models in the active site of the Bcl-2 protein and compounds **4** (a), **5** (b), and **11** (c).

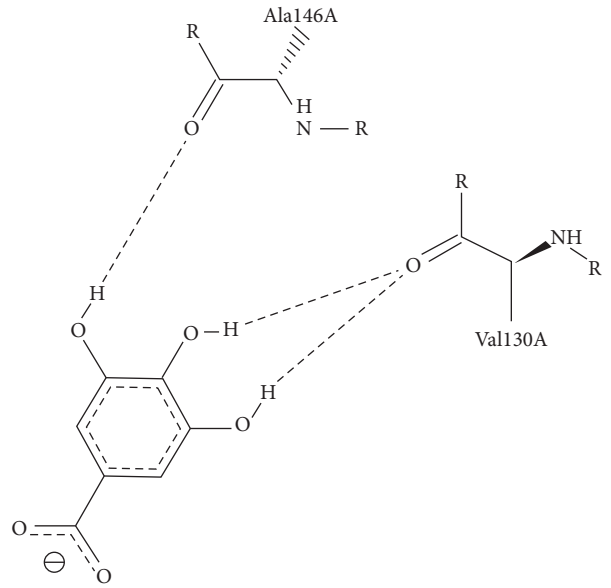


Figure 3: 2D interaction models in the active site of Bcl-2 protein and compound **11.**

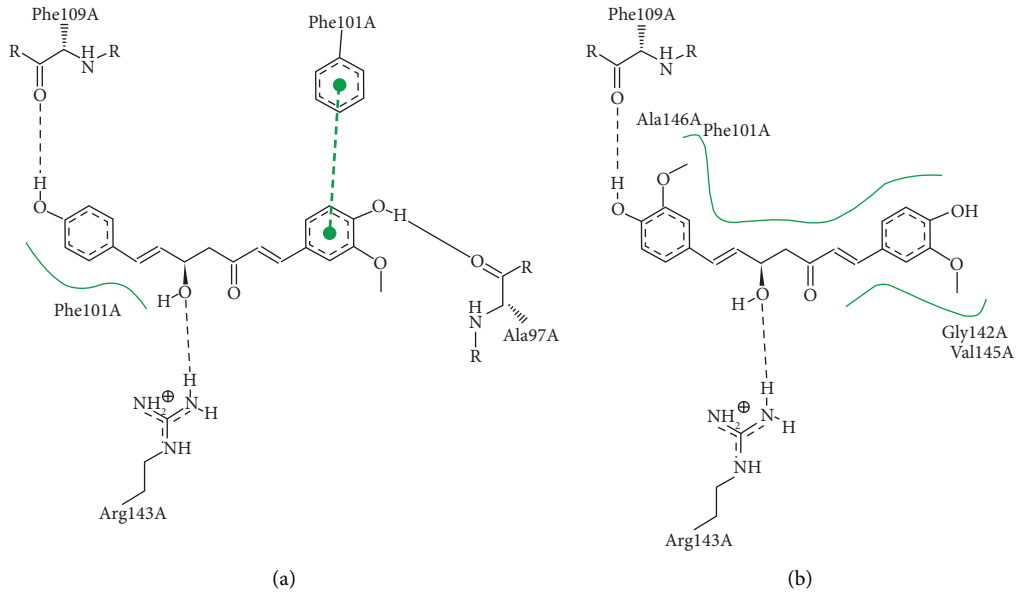


Figure 4: 2D interaction models in the active site of Bcl-2 protein and compounds **4** (a) and **5** (b).

Distinctly, compounds (**4**, **5**, and **11**) possessed potential cytotoxicity against MDA-MB-231, RD, and HepG2 cells with IC_{50} values less than 30 μM, which were demonstrated at the active binding site of the Bcl-2 protein (PDB ID: 2O2F) (Figure 2).

3.5. Molecular Docking Studies for Potential Cytotoxic Compounds. As a result, the docking scores of compounds **4**, **5**, and PAC were −6.31, −6.92, and −6.08 kJ.mol^{-1}, respectively. On the other hand, compound **11** had a low docking score (−3.39 kJ.mol^{-1}) and interacted with the amino acids via the hydrogen bonds formed by Val130 and Ala146 (Figure 3), which are not important.

This model contradicts *in vitro* cytotoxicity in three cancer cell lines, and it implies that **11** displays a different binding pocket for **11** in the Bcl-2 protein and makes a distinction from that of PAC. On the other hand, **4** and **5** interacted through hydrogen bonds with crucial amino acids as follows: Phe109, Arg143, Ala97 (**4**), Phe109, Arg143 (**5**), into the active site of the Bcl-2 protein (Figure 4). The other amino acids had hydrophobic interactions with **4** (Phe101) and **5** (Ala146, Phe101, Gly142, and Val145). Specifically, they formed the same amino acid Arg143 interaction as PAC.

Consequently, binding models **4** and **5** were compatible with their *in vitro* anticancer activity. In addition, these results were further consistent with the *in vitro* inhibitions of these compounds against cell proliferation and the expression of the antiapoptotic protein Bcl-2 [31, 32] or the expression of NO production and proteins of tumor necrosis factor-alpha (TNF-α) [32, 33].

4. Conclusions

The systematic investigation, including sample collection, sample authentication, bioassay-guided separation, and structural characterization of antiproliferative and anti-inflammatory compounds from the Vietnamese herb *Macrosolen tricolor*, was conducted for the first time. Additionally, the *in vitro* cytotoxicity against MDA-MB-231, RD, HepG2, and RAW264.7 cells and anti-inflammation against NO production on LPS-induced inflammation of all fractions and purified metabolites, as well as the *in silico* molecular docking studies of potentially cytotoxic compounds from *M. tricolor*, were detailed for the first time.

A phytochemical study on the active fractions from *M. tricolor* affirmed that the diarylalkanoids (**2**–**5**) present in this species accounted for their anti-inflammatory activities, whereas the main compounds, triterpenoid **1** and phenolic **11**, revealed their cytotoxic properties. Furthermore, molecular docking studies provided evidence to support the good cytotoxicity of compounds **4**, **5**, and **11** as compared with PAC via inhibition of the Bcl-2-regulated apoptotic protein.

The present study proposes that *M. tricolor* plant is a good source of natural antiproliferative and anti-inflammatory agents and supports understanding the ethnopharmacological aspect of *Macrosolen* species in Vietnam.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This research was funded by the Vietnam Academy of Science and Technology (Project no. VAST04.03/22-23).

References

[1] D. Schottenfeld and J. Beebe-Dimmer, "Chronic inflammation: a common and important factor in the pathogenesis of neoplasia," *CA: A Cancer Journal for Clinicians*, vol. 56, no. 2, pp. 69–83, 2006.

[2] S. Tasneem, B. Liu, B. Li, M. I. Choudhary, and W. Wang, "Molecular pharmacology of inflammation: medicinal plants as anti-inflammatory agents," *Pharmacological Research*, vol. 139, no. 1, pp. 126–140, 2019.

[3] P. Tripathi, P. Tripathi, L. Kashyap, and V. Singh, "The role of nitric oxide in inflammatory reactions," *FEMS Immunology and Medical Microbiology*, vol. 51, no. 3, pp. 443–452, 2007.

[4] P. K. S. Uchoa, L. B. de Lima, A. T. A. Pimenta, M. D. C. F. De Oliveira, J. Mafezoli, and M. A. S. Lima, "HPLC quantification of cytotoxic compounds from Aspergillus Niger," *Journal of Chemistry*, vol. 2017, Article ID 6969358, 7 pages, 2017.

[5] G. Mouthé Happi, M. Towa Yimtchui, S. A. Ahmed et al., "Chemical constituents from *Uapaca guineensis* (Phyllanthaceae), and the computational validation of their anti-leishmanial and anti-inflammatory potencies," *Journal of Chemistry*, vol. 2022, Article ID 7087110, 21 pages, 2022.

[6] T. N. Thi Pham, T. T. Nguyen, T. Le Thi Nguyen et al., "Antioxidant and anti-inflammatory activities of phytochemicals from Ruellia tuberosa," *Journal of Chemistry*, vol. 2022, Article ID 4644641, 14 pages, 2022.

[7] K. H. Le, T. K. N. Bui, N. K. T. Pham et al., "Macrotricolorin A, a new diarylpropanoid from the vietnamese plant macrosolen tricolor (lecomte) danser," *Natural Product Research*, vol. 36, no. 1, pp. 165–170, 2022.

[8] K. H. Le, N. M. Phan, T. D. Bui et al., "Phytochemical components of the whole plant of macrosolen tricolor (L.) Dans," *Vietnam Journal of Chemistry*, vol. 59, no. 3, pp. 326–330, 2021.

[9] X. G. Vu, *Study on biological characteristic, chemical constituents and biological activities of taxillus chinensis (dc.) dans. and macrosolen tricolor (l.) dans*, Ph.D. Thesis, Hanoi Medical University, Hanoi, Vietnam, 2014.

[10] X. G. Vu, T. K. Pham, T. T. Nguyen, and T. V. A. Pham, "Study on hepatoprotective activity of taxillus chinensis (dc) dans. and macrosolen tricolor (lecomte) dans," *Vietnam Pharmacy Journals*, vol. 426, no. 10, pp. 40–43, 2011.

[11] X. G. Vu, T. K. Pham, T. T. Nguyen, and T. V. A. Pham, "Study on anti-inflammatory activity of Taxillus chinensis (DC) dans. and macrosolen tricolor (lecomte) dans," *Vietnam Pharmacy Journals*, vol. 425, no. 9, pp. 41–45, 2011.

[12] T. Phan, T. K. Pham, and V. K. Phan, "Isolation and elucidation triterpenoids from the aerial part of macrosolen tricolor (lecomte) dans," *Journal of Portuguese Linguistics*, vol. 12, no. 1, pp. 41–48, 2013.

[13] D. Tri Mai, T. Nghia Ngo, N. T. Ly Nguyen et al., "Cytotoxic activity and phytochemical composition of stereospermum binhchauensis v.s. dang leaves," *Natural Product Research*, vol. 35, no. 4, pp. 641–645, 2021.

[14] B. L. C. Huynh, N. K. T. Pham, and T. P. Nguyen, "Vinapraesorediosic acids D and E from the lichen Parmotrema praesorediosum (nyl.) hale," *Phytochem Phytochemistry Letters*, vol. 41, no. 1, pp. 61–64, 2021.

[15] N. M. Phan, T. H. T. Do, L. T. T. Nguyen et al., "Hepatoprotection and phytochemistry of the vietnamese herbs cleome chelidonii and cleome viscosa stems," *Journal of Chemistry*, vol. 2021, Article ID 5578667, 2021.

[16] K. H. Le, B. L. C. Huynh, N. K. T. Pham et al., "Cycloartane-type triterpenoids from the whole plants of Macrosolen bidoupensis," *Journal of Asian Natural Products Research*, vol. 24, no. 6, pp. 596–602, 2022.

[17] K. H. Le, T. C. Nguyen, N. D. P. Nguyen et al., "Cytotoxic activity and phytochemical constituents of Macrosolen bidoupensis Tangane & V.S. Dang," *Records of Natural Products*, vol. 15, no. 1, pp. 71–75, 2020.

[18] K. H. Le, D. T. Phan, N. M. Phan et al., "Flavonoids and phenolics from the whole plant of macrosolen bidoupensis tagane & v.s. dang," *Vietnam Journal of Chemistry*, vol. 59, no. 1, Article ID 22021, 115–119 pages, 2021.

[19] K. H. Le, B. L. C. Huynh, N. K. T. Pham et al., "Triterpenoids from the Macrosolen bidoupensis Tagane & V.S. Dang whole plant," *Vietnam Journal of Chemistry*, vol. 59, no. 4, pp. 475–479, 2021.

[20] F. Denizot and R. Lang, "Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability," *Journal of Immunological Methods*, vol. 89, no. 2, pp. 271–277, 1986.

[21] M. Bruncko, T. K. Oost, B. A. Belli et al., "Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-xL," *Journal of Medicinal Chemistry*, vol. 50, no. 4, pp. 641–662, 2007.

[22] G. M. Morris, R. Huey, W. Lindstrom et al., "Autodock4 and autodocktools4: automated docking with selective receptor flexibility," *Journal of Computational Chemistry*, vol. 30, no. 16, pp. 2785–2791, 2009.

[23] R. A. Laskowski and M. B. Swindells, "LigPlot+: multiple ligand-protein interaction diagrams for drug discovery," *Journal of Chemical Information and Modeling*, vol. 51, no. 10, pp. 2778–2786, 2011.

[24] F. Pereira Beserra, M. Xue, G. Maia, A. Leite Rozza, C. Helena Pellizzon, and C. J. Jackson, "Lupeol, a pentacyclic triterpene, promotes migration, wound closure, and contractile effect *in vitro*: possible involvement of PI3K/Akt and p38/ERK/MAPK pathways," *Molecules*, vol. 23, no. 11, p. 2819, 2018.

[25] M. Shaaban, M. Ali, M. F. Tala, A. Hamed, and A. Z. Hassan, "Ecological and phytochemical studies on euphorbia retusa (forssk.) from egyptian habitat," *Journal of Analytical Methods in Chemistry*, vol. 2018, Article ID 9143683, 2018.

[26] G. Tsasi, P. Samara, O. Tsitsilonis, G. Juergenliemk, and H. Skaltsa, "Isolation, identification and cytotoxic activity of triterpenes and flavonoids from green walnut (Juglans regia L.) Pericarps," *Records of Natural Products*, vol. 10, no. 1, pp. 83–92, 2016.

[27] Y. Okada, A. Omae, and T. Okuyama, "A new triterpenoid isolated from lagerstronemia speciosa (l.) pers," *Chemical and Pharmaceutical Bulletin*, vol. 51, no. 4, pp. 452–454, 2003.

[28] S. I. Uehara, I. Yasuda, K. Takeya, H. Itokawa, and Y. Iitaka, "New bisabolane sesquiterpenoids from the rhizomes of curcuma xanthorrhiza (zingiberaceae)," *Chemical and Pharmaceutical Bulletin*, vol. 37, no. 1, pp. 237–240, 1989.

[29] J. A. Quincoces Suarez, D. G. Rando, R. P. Santos et al., "New antitumoral agents i: in vitro anticancer activity and in vivo acute toxicity of synthetic 1, 5-bis(4-hydroxy-3-methoxyphenyl)-1, 4-pentadien-3-one and derivatives," *Bioorganic & Medicinal Chemistry*, vol. 18, no. 17, pp. 6275–6281, 2010.

[30] D. C. Burns, D. A. Ellis, and R. E. March, "A predictive tool for assessing (13)C NMR chemical shifts of flavonoids," *Magnetic Resonance in Chemistry*, vol. 45, no. 10, pp. 835–845, 2007.

[31] Z. J. Yang, S. Y. Huang, D. D. Zhou et al., "Effects and mechanisms of curcumin for the prevention and management of cancers: an updated review," *Antioxidants*, vol. 11, no. 8, 1481 pages, 2022.

[32] S. K. Sandur, M. K. Pandey, B. Sung et al., "Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism," *Carcinogenesis*, vol. 28, no. 8, pp. 1765–1773, 2007.

[33] L. J. Zhang, C. F. Wu, X. L. Meng et al., "Comparison of inhibitory potency of three different curcuminoid pigments on nitric oxide and tumor necrosis factor production of rat primary microglia induced by lipopolysaccharide," *Neuroscience Letters*, vol. 447, no. 1, pp. 48–53, 2008.