CYTOTOXICITY OF COMPOUNDS FROM THE WHOLE PLANT OF MACROSOLEN TRICOLOR AGAINST HEPG2 CELL LINE

Đến tòa soạn 10-03-2021

Ngo Trong Nghia, Dang Thi Hong Ngoc
Kien Giang University
Huynh Bui Linh Chi
Dong Nai University
Pham Nguyen Kim Tuyen
Sai Gon University
Phan Nhat Minh, Bui Trong Dat, Mai Dinh Tri, Nguyen Tan Phat
Graduate University of Science and Technology, Vietnam Academy of Science and Technology
Institute of Chemical Technology, Vietnam Academy of Science and Technology

TÓM TẮT

HOẠT TÍNH GÂY ĐỘC TẾ BÀO UNG THƯ GAN (HEPG2) CỦA CÁC HỢP CHẤT TỪ CÂY ĐẠI CÁN TAM SẮC (MACROSOLEN TRICOLOR (LEC.) DANS.)

*Bằng các phương pháp sắc ký thường quy, bốn hợp chất (**1**-**4**) đã được phân lập từ cao ethyl acetate của cây Đại cán tam sắc là 4-hydroxybenzoic aicd (**1**), 3,4,5-trimethoxybenzoic acid (**2**), 3-methylcatechol (**3**), luteolin (**4**). Cấu trúc của hợp chất được xác định bằng các phương pháp phổ hiện đại như: 1D & 2D-NMR, và HRMS. Các hợp chất (**1**-**4**) được đánh giá hoạt tính gây độc tế bào ung thư gan HepG2 theo phương pháp SRB. Kết quả cho thấy hợp chất (**3**) và (**4**) có khả năng ức chế dòng tế bào ung thư gan HepG2 với giá trị IC_{50} lần lượt là 27,08 ± 3,83 µg/mL và 54,86 ± 2,36 µg/mL.*
Từ khóa: *Macrosolen tricolor*, Cytotoxicity, *HepG2, Loranthaceae.*

1. INTRODUCTION

Macrosolen tricolor (Lec.) Dans. is a traditional medicinal plant and belongs to the family Loranthaceae, which is distributed in Nha Trang, and Phan Rang[1,2]. Pharmacological investigations of extracts from *M. tricolor* expressed anti-inflammatory, α-glucosidase inhibition, and hepatoprotective effects[2-4]. Phytochemical studies on this species found triterpenoid, flavonoid, and phenolic compounds[3-6]. Since it is useful in traditional medicine, as a part of our ongoing research to discover new antitumor compounds from natural resources led to the phytochemical investigation of this plant. In our earlier research, from the whole plant of *M. tricolor* of Viet Nam, diarylheptanoids, phenolics, flavonoids, steroids, and triterpenoids were isolated[6,7]. This paper describes the isolation, structural elucidation, and cytotoxic activity of compounds from the ethyl acetate extract of the whole plant of *M. tricolor* (Lec.) Dans.

2. EXPERIMENTAL

2.1. Plant material

The whole plant of *M. tricolor* was collected in Ba Ria Vung Tau province, Vietnam, and identified by Dr. Van Son Dang, Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh city. Then, the whole plants of *M. tricolor* were dried, powdered (4.3 kg).

2.2. Equipment and chemicals

The high resolution electrospray ionisation mass spectrometry (HR-ESI-MS) was recorded on Bruker MicrQTOF-QII spectrometer. The 1D and 2D NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer. Column chromatography was carried out using silica gel (230-400 mesh). TLC was carried out in silica gel plates (Kieselgel 60 F254, Merck). Ethanol, methanol, *n*-hexane, ethyl acetate, chloroform (Chemsol). Trichloroacetic acid, acetic acid, dimethyl sulfoxide (Merck). Tris base (Promega). Sulforhodamine B (Sigma). Camptothecin (Calbiochem). HepG2 were purchased from the American Type Culture Collection (Manassas, Rockville).

2.3. Methods

2.3.1. Extraction and isolation

The whole plants of *M. tricolor* were dried, powdered (4.3 kg), and extracted with EtOH 96% at room temperature for three times to make the crude extract. This extract (1100 g) was subjected to liquid-liquid extraction, and separated into *n*-hexane (100 g), and ethyl acetate (106 g) extracts. The EtOAc extract (106 g) was separated on a silica gel column eluting with *n*-hexane-ethyl acetate-methanol (50-75-0→0-90-10, v/v/v) to yield four fractions (MTE.I-IV). Fraction MTE.I (30 g) was applied on column chromatography with mobile phase *n*-hexane-EtOAc (4–1 to 1–9, v/v) to obtain ten subfractions (MTE.I.1–MTE.I.10),. Subfraction MTE.I.1 (2.4 g) was purified on silica gel with $CHCl_3$–MeOH (99:1 to 95:5, *v*/*v*) to give (**2)** (8 mg). Subfraction MTE.I.2 (3.5 g) was purified onsilica gel column with $CHCl_3$–MeOH solvent system (98:2 to 95: 5, v/v) to afford (**1)** (10 mg) and **(3)** (9 mg)**.** Subfraction MTE.I.4 (2.6 g) was applied on column chromatography eluting with

$CHCl_3$–MeOH system (98:2 to 90:10, v/v) to afford **(4)** (8 mg).

2.3.2. Biological assay

Biological assay (SRB assay) described by Dr. Nguyen Thi My Nuong[8]. Cells were seeded in 96-well plates for 24 h before being incubated with sample for 48 h. Treated cells were fixed with cold 50 % (w/v) trichloroacetic acid solution for 1–3 h, washed, and stained with 0.2 % (w/v) SRB for 20 min. After five washes with 1 % acetic acid, protein-bound dye was solubilized in 10 mM Tris base solution. Optical density values were determined at the wavelengths of 492 nm and 620 nm. The percentage of growth inhibition (Inh %) was calculated according to the formula: Inh % = (1-[ODt/ODc] x 100) %, in which ODt and ODc are the optical density value of the test sample and the control sample, respectively. Camptothecin was used as a positive control.

3. RESULTS AND DISCUSSION

Compound (**1)** was obtained as a white amorphous powder. HR-ESI-MS $[M-H]^-$ = 137.0253, calcd. 137.0239. ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 7.90 (2H, *dd*, *J* = 2.0, 8.0 Hz, H-2, H-6), 6.84 (2H, *dd*, *J* = 2.0, 8.0 Hz, H-3, H-5). Phổ ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 122.7 (C-1), 133.0 (C-2), 116.0 (C-3), 163.3 (C-4), 116.0 (C-5), 133.0 (C-6), 170.1 (-COOH).

Compound (**2)** was obtained as a white amorphous powder. HR-ESI-MS $[M-H]^-$ = 211.0616, calcd. 211.0606. ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 3.85 (3H, *br s*, 3-OCH_3), 3.90 (6H, *br s*, 4-OCH_3, 5-OCH_3), 7.36 (2H, *s*, H-2, H-6. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 127.2 (C-1), 108.2 (C-2), 154.3 (C-3, C-5), 143.4 (C-4), 108.2 (C-6); 56.7 (C-7), 61.1 (C-8), 56.7 (C-9), 169.4 (-COOH).

Compound (**3)** was obtained as a white amorphous powder. HR-ESI-MS $[M-H]^-$ = 123. 0453, calcd. 123.0446. ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 6.56 (1H, *d*, *J* = 6.5 Hz, H-6), 6.58 (1H, *d*, *J* = 3.5 Hz, H-4), 6.63 (1H, *t*, *J* = 7.0 Hz, H-5), 2.20 (3H, *s*, -CH_3). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 145.9 (C-1), 144.4 (C-2), 125.9 (C-3), 113.7 (C-5), 122.7 (C-4), 120.2 (C-6), 16.0 (C-7).

Compound (**4)** was obtained as a yellow amorphous powder. ^{1}H-NMR (500 MHz, DMSO-d_6): 6.65 (1H, *s*, H-3), 6.18 (1H, *d*, *J* = 2.0 Hz, H-6), 6.44 (1H, *d*, *J* = 2.0 Hz, H-8), 7.39 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.89 (1H, *d*, *J* =

8.0 Hz, H-5′), 7,41 (1H, *dd, J* = 2.0, 8.5 Hz, H-6′)**,** 12.96 (1H, *s*, OH-5). ^{13}C-NMR (125 MHz, DMSO-d_6): 163.9 (C-2), 102.8 (C-3), 181.6 (C-4), 161.5 (C-5), 98.8 (C-6), 164.2 (C-7), 93.8 (C-8), 157.3 (C-9), 103.6 (C-10), 121.4 (C-1′), 113.3 (C-2′), 145.7 (C-3′), 149.8 (C-4′), 116.0 (C-5′), 119.0 (C-6′).

The cytotoxicity of extracts and all isolated compounds were evaluated against HepG2 cell line by SRB assay and is presented in table 1.

Table 1. Cytotoxic activity of all fractions and isolated compounds

No.	Sample	Concentration (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)
1	ethanol extract	100	34.90 ± 5.57	
2	*n*-hexane extract	100	18.10 ± 4.38	
3	ethyl acetate extract	100	62.82 ± 1.83	
4	**(1)**	100	-12.36 ± 6.48	
5	**(2)**	100	-5.05 ± 4.64	
6	**(3)**	100	91.22 ± 1.78	27.08 ± 3.83
7	**(4)**	100	71.20 ± 2.46	54.86 ± 2.36
8	Camptothecine	0.07	50.84 ± 2.03	0.79 0.023

Compound **(1)**: The molecular formula was established as $C_7H_6O_3$ by HR-ESI-MS $[M-H]^-$ = 137.0253, calcd. 137.0239. The ^{1}H-NMR spectra displayed four aromatic methine protons at δ_H 7.90 (2H, *dd, J* = 2.0, 8.0 Hz, H-2, H-6) and 6.84 (2H, *dd, J* = 2.0, 8.0 Hz, H-3, H-5). The ^{13}C-NMR data of **(1)** described seven carbons including one carbonyl carbon at δ_C 170.1 (COOH), four aromatic methine carbons at δ_C 133.0 (C-2), 116.0 (C-3), 116.0 (C-5), 133.0 (C-6), one oxygenated aromatic carbon at δ_C 163.3 (C-4), and one quaternary aromatic carbon at δ_C 122,7 (C-1). Thus **(1)** had a 1,4-disubstituted benzene ring bearing one hydroxyl and one carboxyl group. Based on data of HR-MS, NMR and compared with published data, the structure of **(1)** was identified as 4-hydroxybenzoic acid[9].

Compound **(2):** The molecular formula was established as $C_{10}H_{12}O_5$ by HR-ESI-MS $[M-H]^-$ = 211.0616, calcd. 211.0606. The ^{1}H-NMR data of **(2)** exhibited two aromatic methine protons at δ_H 7.36 (2H, *s*, H-2 & H-6), and nine methoxy protons at δ_H 3.85 (3H, *brs*, 3-OCH_3) and 3.90 (6H, *brs*, 4-OCH_3, 5-OCH_3). The ^{13}C-NMR spectrum of **(2)** showed ten carbons including one carbonyl carbon at δ_C 169.4 (-COOH), two aromatic methine carbons at δ_C 108.2 (C-2, C-6), three oxygenated aromatic carbons at δ_C 143.4 (C-4), 154.3 (C-3, C-5), one quaternary aromatic carbon at δ_C 127.2 (C-1), and three methoxy groups at δ_C 56.7 (C-7, C-9), 61.1 (C-8). Thus, **(2)** was a benzoic acid derivative. The HMBC spectrum of **(2)** showed the methoxy protons at δ_H 3.85 (3H, *brs*, 3-OCH_3) correlated with carbon at 154.3 (C-3); 3.90 (6H, *brs*, 4-OCH_3, 5-OCH_3) correlated with two carbons at δ_C 143.4 (C-4),154.3 (C-5), respectively. Based on data of HR-MS, NMR and compared with published data, the structure of **(2)** was indicated 3,4,5-trimethoxybenzoic acid [9].

Compound **(3)**: The molecular formula was established as $C_7H_8O_2$ by HR-ESI-MS $[M-H]^-$ = 123.0453, calcd. 123.0446. The ^{1}H-NMR data of **(3)** revealed three aromatic methin protons at δ_H 6.56 (1H, *d, J* = 6.5 Hz, H-6), 6.58 (1H, *d, J* = 3.5 Hz, H-4), 6.63 (1H, *t, J* = 7.0 Hz, H-5), and three methyl protons at δ_H 2.20 (3H, *s*, -CH_3). The ^{13}C-NMR spectrum of **(3)** showed seven carbons including one methyl carbon at δ_C 16.0 (C-7), three aromatic carbons at δ_C 113.7 (C-5), 120.2 (C-6), 122.7 (C-4), one quaternary aromatic carbon at δ_C 125.9 (C-3), and two oxygenated aromatic carbon at δ_C 144.4 (C-2), 145.9 (C-1). Thus **(3)** had a 1,2,3-trisubstituted benzene ring bearing two hydroxyl and one methyl groups. The HMBC showed three methyl protons at δ_H 2.20

(3H, *s*, -CH_3) correlated with four carbons at δ_C δ_C 125.9 (C-3), 144.4 (C-2), 113.7 (C-5), and 122.7 (C-4), which determined the positions of the methyl group. Based on data of HR-MS, NMR and compared with published data, the structure of **(3)** was indicated 3-methylcatechol[9,10].

Compound **(4):** The 13C-NMR spectra of (**4**) corresponed to fifteen carbons of a flavone skeleton, including one carbonyl carbon at δ_C 181.6 (C-4), six oxygenated aromatic carbons at δ_C 163.9 (C-2), 161.5 (C-5), 164.2 (C-7), 157.3 (C-9), 145.7 (C-3'), and 149.8 (C-4'), two quaternary aromatic carbons at δ_C 103.6 (C-10) and 121.4 (C-1′), and six aromatic methine carbons at δ_C 102.8 (C-3), 98.8 (C-6), 93.8 (C-8), 113.3 (C-2′), 116.0 (C 5′), 119.0 (C-6′). The ^{1}H-NMR data of (**4**) exhibited two *meta* coupled protons at δ_H 6.18 (1H, *d*, *J* = 2.0 Hz, H-6) and 6.44 (1H, *d*, *J* = 2.0 Hz, H-8), one aromatic methine proton at δ_H 6.66 (1H, *s*, H-3), one hydroxyl proton at δ_H 12.96 (1H, *s*, OH-5). In addition, the presence of ABX system at δ_H 7.39 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.41 (1H, *dd*, *J* = 2.0, 8.5 Hz, H-6') and 6.89 (1H, *d*, *J* = 8.0 Hz, H-5') which indicated a luteolin framework. The HSQC and HMBC experiments supported the structure of **(4)**. Based on data of NMR and compared with published data, the structure of, the structure of **4** was identified luteolin[11].

For the first time, 4-hydroxybenzoic acid, 3,4,5-trimethoxybenzoic acid, luteolin, and 3-methylcatechol, were isolated from *M. tricolor*, while macrotricolorin A, curcumin, demethoxycurcumin, bisdemethoxycurcumin, kaempferol, vitexin, orientin, gallic acid, 3,3'-dimethoxyellagic acid, 3,3′ dimethoxyellagic acid 4-*O*-*β*-D xylopyranoside, stigmast-5-ene 3-*O*-*β*-D-glucopyranoside, stigmast-5-ene 3-*O*-*β*-D-(6'-*O*-margaroyl)glucopyranoside, 20(29)-lupene-3*β*-nonadecanoyl-7*β*,15*α*-diol, 20(29)-lupene-3*β*-nonadecanoyl-7*β*-ol, and lupeol have been reported from this plant in Vietnam[2-7].

The cytotoxicity of the extracts from the whole plants of *Macrosolen tricolor* were evaluated against HepG2 human liver cancer cell line (Table 1). At the concentration of 100 µg/mL the ethyl acetate extract expressed moderate cytotoxic effect on HepG2 cell line (Inh (%) 62.82%, respectively). Therefore, the ethyl acetate extract was further investigated for its phytochemical constituents. The cytotoxic activities of four compounds (**1-4**) were examined against HepG2 cell line (Table 1). Compounds (**3**) and (**4**) showed meaningful cytotoxicity against HepG2 cell line (IC_{50} of 27.08 and 54.86 µg/mL, respectively) whereas, compounds (**1**) and **(2)** did not inhibit the growth of HepG2 cell line (Inh (%) of -12.36 and -5.05, respectively). Thus, compounds (1) and (2) did not determined IC_{50} value. According to these results, compounds **(3)** and **(4)** contributed as the components that accounted for the cytotoxic effect of the EtOAc extract from *M. tricolor* (Lec.) Dans against HepG2 cell line.

4. CONCLUSION

From the ethyl acetate extract of the *M. tricolor* (Lec.) Dans whole plants, four compounds, 4-hydroxybenzoic acid **(1)**, 3,4,5-trimethoxybenzoic acid **(2)**, 3-methylcatechol **(3)**, and luteolin (**4**) were isolated by chromatographic methods. Compounds (**1-4**) were isolated from this plant for the first time. Cytotoxicity assay result showed that compounds **(3)**, and **(4)** expressed moderate cytotoxicity against HepG2 cell line with IC_{50} of 27.08 and 54.86 µg/mL, respectively, whereas the other compounds showed no activities.

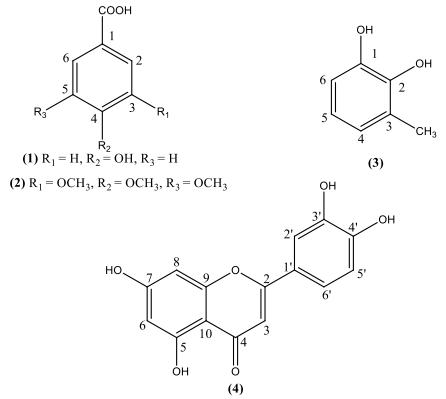

*Figure 1. Chemical structures of compounds **1-4***

Acknowledgment: This research is funded by Kien Giang University under *g*rant number*: A2020-TNMT-07*

REFERENCES

1. Pham H. H. An illustrated flora of Vietnam, *Tre Publishing House-Hanoi*, 2000, *II*, 129-131.

2. Ha T.B.N. Investigation and research on some Vietnamese plants that support the regulation of blood sugar for use in patients with type 2 diabetes mellitus, 2012, *PhD Thesis in Biology, VNU University of Science, Hanoi*.

3. Vu X. G., Pham T. K., Nguyen T. T., Pham T. V. A., Study on hepatoprotective activity of *Taxillus chinensis* (DC) Dans. and *Macrosolen tricolor* Dans, *Vietnamese Pharmaceutical Journal*, 2011, *426*, 40-43.

4. Vu X. G., Pham T. K., Nguyen T. T., Pham T. V. A. Study on anti-inflammatory activity of *Taxillus chinensis* (DC) Dans. and *Macrosolen tricolor* (Lecomte) Dans, *Vietnamese Pharmaceutical Journal*, 2011, *425*, 41-45.

5. Vu X. G., Pham T. K., Phan V. K., Isolation and elucidation triterpenoids from the aerial of *Macrosolen tricolor* (Lecomte) Dans, *Vietnamese Pharmaceutical Journal*, 2013, *450*, 41-48.

6. Le K. H., Bui T. K. N., Pham N. K. T., Dang V. S., Ngo T. N., Phan N. M., Bui T. D., Tran D. T., Nguyen Q. H., Ngo Q. L., Mai D. T., Nguyen T. P. Macrotricolorin A, a new diarylpropanoid from the Vietnamese plant *Macrosolen tricolor* (Lecomte) Danser, *Natural Products Research*, 2020, 1-6 (DOI:10.1080/14786419.2020.1771710).

7. Le K. H., Phan N. M., Bui T. D., Ngo Q. L., Huynh B. L. C., Pham N. K. T., Phan H. L., Tran T. N. M., Mai D. T., Nguyen T. P. Phytochemical components of *Macrosolen tricolor* (L.) Dans. whole plant, *Vietnam Journal of Chemistry*, 2021, (DOI: 10.1002/vjch.202000173).

8. Nguyen M.N.T., Ho-Huynh T.D., Selective cytotoxicity of a Vietnamese traditional formula, Nam Dia long, against MCF-7 cells by synergistic effects. *BMC Complementary and Alternative Medicine*, 2016, *16*, 220.

9. Vu D. L., Pham G. N., Hoang V. H, Nguyen T. P., Extraction and Separation of Various Compounds from Leaf of *Leea Rubra* Blume ex Spreng, *VNU Journal of Science, Medical and Pharmaceutical Sciences*, 2016, **32** (1), 12-17.

10. Bishwajit S., Parinita B., A new avenue to dakin reaction in H_2O_2–Wersa, *RSC Advances*, 2015, **5** (128), 105583- 105586.

11. Lee S., Choi M. J., Kim H. Y., Cho E. J., Flavonoids from *Taraxacum coreanum* protect from radical-induced oxidative damage, *Journal of Medicinal Plants Research*, 2012, **6** (40), 5377-5384.