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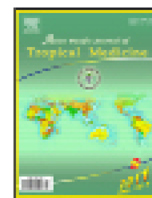

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Anti-inflammatory effects of fatty acids isolated from *Chromolaena odorata*

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ABSTRACT

Objective: To identify inhibitors of nitric oxide production and NF-κB activity from *Chromolaena odorata* (*C. odorata*). **Methods:** The compounds isolated from the aerial parts of *C. odorata* by bioassay-guided fractionation were investigated for their inhibitory effects on the NO production and NF-κB activity in LPS-stimulated RAW264.7 cells. **Results:** Six fatty acids (*S*)-coriolic acid (1), (*S*)-coriolic acid methyl ester (2), (*S*)-15,16-didehydrocoriolic acid (3), (*S*)-15,16-didehydrocoriolic acid methyl ester (4), linoleamide (5) and linolenamide (6) were isolated. All compounds inhibited the NO production at concentrations consistent with those required for NF-κB inhibition. Compound 2 was the most active with the IC_{50} values of 5.22 and 5.73 μM. The addition of a double bond in the fatty chain decreased the inhibitory effects while the methyl esterification increased the activities. **Conclusions:** The fatty acid components in *C. odorata* with NF-κB inhibitory activity could explain the anti-inflammation property of this plant in traditional medicine. This study could also contribute to the better use of *C. odorata* for human health care.

1. Introduction

Chromolaena odorata (L.) (*C. odorata*) King and Robinson, formerly named *Eupatorium odoratum* (Asteraceae) has been used in Vietnamese traditional medicine as hemostatic, anti-inflammation drugs and for treatment of intestine diseases and burn wounds[1]. Previous studies showed that this plant contains essential oil, alkaloids and flavonoids[2-4], together with interesting biological activities including antibacteria, anti-inflammation and antioxidant, cytotoxicity[5-9]. In Vietnam, the National Institute of Burns have approved an ointment (commercial name as Eupolin) from the the aqueous extract of *C. odorata* for burns treatment[10]. Nitric oxide (NO) has been known to involve in the regulation of various physiological processes in mammals and the overproduction of NO is responsible for the pathological development of inflammation, cancer, and diabetes[11]. The production of NO is primary regulated by the nuclear transcription factor-κB (NF-κB), a member of the Rel domain-containing proteins relating to multiple pathophysiological conditions such as cancer, arthritis, asthma, inflammatory bowel disease and other inflammation[12]. In our continuing search for anti-inflammatory agents of natural origin, we found that the methanol extract of the *C. odorata* showed strong inhibitory effect on the production of NO and NF-κB activation in LPS-induced RAW264.7 cells. The phytochemical investigation of the methanol extract of the *C. odorata* led to the isolation of six fatty acid derivatives (*S*)-coriolic acid (1), (*S*)-coriolic acid methyl ester (2), (*S*)-15,16-didehydrocoriolic acid (3), (*S*)-15,16-didehydrocoriolic acid methyl ester (4), linoleamide (5) and linolenamide (6) (Figure 1). The present paper describes the isolation and the inhibitory effects of the isolated compounds on the NO production and NF-κB activation.

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2. Materials and methods

2.1. General experimental procedures

Optical rotation was measured on a JASCO P-2000 polarimeter. ^{1}H- and ^{13}C-NMR were performed on a Bruker AM500 FT-NMR Spectrometer using TMS as internal standard. ESI-MS spectra were recorded on an Agilent 1200 Series LC-MSD Ion Trap.

2.2. Plant materials

Aerial parts of *C. odorata* were collected in Thach Thanh, Thanh Hoa, Vietnam in January 2010 and were identified by Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam. A voucher specimen (No. 20100120) was deposited in the herbarium of the Department of Bioactive Products, Institute of Marine Biochemistry, VAST, Vietnam.

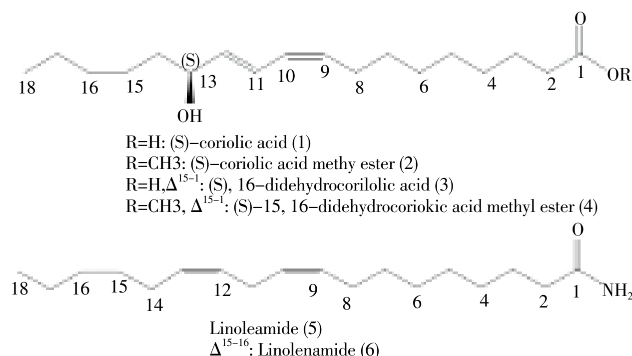

Figure 1. Structure of compounds 1-6 isolated from *C. odorata*.

2.3. Extraction and isolation

The air-dried and powdered aerial parts of *C. odorata* (5 kg) were extracted with methanol [(3×10) L]. The MeOH extract was concentrated and the residue (180 g) was partitioned in chloroform/water. The chloroform soluble extract was concentrated in vaccuo to give a brown solid (110 g), which was then chromatographed on silica gel column using a chloroform-methanol gradient as mobile phase to afford eight fractions A1-A8. Of these, A4 was subjected on silica gel column with *n*-hexane-acetone (5:1 v/v) as eluant to afford 2 (14 mg) and 4 (4.5 mg) together with four fractions B1-B4. From B4, 5 (6.2 mg) and 6 (11.5 mg) were eluted through a silica gel column by *n*-hexane-acetone (4:1). Fraction A5 was chromatographed on silica gel with *n*-hexane- acetone (3:1 v/v) affording 5 subfractions C1-C5. The C3 and C4 were purified on reverse phase RP-18 column using methanol-water (5： 1) as eluant to give 1 (22 mg) and 3 (15 mg).

(*S*)-Coriolic acid (1): yellow oil. $[\alpha]_D^{20}$: +3.8 (*c* 0.1, $CHCl_3$). ESI-MS *m/z* 295.6 $[M-H]^-$. ^1H- and ^{13}C-NMR, see Table 1 and 2.

(*S*)-Coriolic acid methyl ester (2): yellow oil. $[\alpha]_D^{20}$: +12.5 (*c* 0.1, $CHCl_3$). ESI-MS *m/z* 331.5 $[M+Na]^+$. ^1H- and ^{13}C-NMR, see Table 1 and 2.

(*S*)-15,16-didehydrocoriolic acid (3): yellow oil. $[\alpha]_D^{20}$: +29.4 (*c* 0.1 $CHCl_3$). ESI-MS *m/z* 293.5 $[M-H]^-$. ^1H- and ^{13}C-NMR, see Table 1 and 2.

(*S*)-15,16-didehydrocoriolic acid methyl ester (4) yellow oil. $[\alpha]_D^{20}$: +32.1 (*c* 0.1 $CHCl_3$). ESI-MS *m/z* 333.5 $[M+Na]^+$. ^1H- and ^{13}C-NMR, see Table 1 and 2.

Linoleamide (5): yellow oil. ESI-MS *m/z* 278.5 $[M-H]^-$. ^1H- and ^{13}C-NMR, see Table 1 and 2.

Linolenamide (6): yellow oil. ESI-MS *m/z* 276.5 $[M-H]^-$. ^1H- and ^{13}C-NMR, see Table 1 and 2.

2.4. Measurement of NO production

Murine macrophage RAW264.7 cells were seeded in 96-well plate at 2×10^5 cells/well and incubated for 3 h. The plate was pretreated with various concentrations of MB for 30 min and then incubated for another 24 h with or without 1 μg/mL LPS. As a parameter of NO synthesis, nitrite concentration in the culture supernatant was measured by the Griess method[13]. 100 μL of the culture supernatant were transferred to other 96-well plate and 100 μL of Griess reagent were added. The absorbance of the reaction solution was read at 570 nm with a microplate reader (Molecular Devices Co., Menlo Park, CA). The remaining cell solutions in cultured 96-well plate were used to evaluate cell viability by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Table 1
^1H NMR (500 MHz, $CDCl_3$) data of compound 1-6.

No	1	2	3	4	5	6
1	-	-	-	-	-	-
2	2.34 (2H, t, 7.6)	2.30 (2H, t, 7.6)	2.36 (2H, t, 7.6)	2.36 (2H, t, 7.6)	2.22 (2H, t, 10.0)	2.18(2H, t, 9.6)
3	1.63 (2H, m)	1.63 (2H, m)	1.63 (2H, m)	1.62 (2H, m)	1.55 (2H, m)	1.58 (2H, m)
4-7	1.26-1.37 (m)	1.26-1.37 (m)	1.26-1.47 (m)	1.26-1.47 (m)	1.20-1.40 (m)	1.24-1.40 (m)
8	2.17 (2H, m)	2.18 (2H, m)	2.18 (2H, m)	2.18 (2H, m)	2.07 (2H, m)	2.07 (2H, m)
9	5.44 (1H, m)	5.43 (1H, m)	5.44 (1H, m)	5.44 (1H, m)	5.36 (m)	5.32 (m)
10	5.97 (1H, t, 10.7)	5.97 (1H, t, 11.2)	5.98 (1H, t, 10.7)	5.98 (1H, t, 11.2)	5.36 (m)	5.32 (m)
11	6.49 (1H, dd, 10.7, 15.4)	6.48 (1H, dd, 11.2, 15.2)	6.52 (1H, dd, 10.7, 15.4)	6.52 (1H, dd, 10.8, 15.2)	2.76 (2H, t, 7.6)	2.79 (2H, t, 7.6)
12	5.66 (1H, dd, 15.4, 6.5)	5.66 (1H, dd, 15.2, 6.8)	5.69 (1H, dd, 15.4, 6.5)	5.68 (1H, dd, 15.2, 6.8)	5.36 (m)	5.32 (m)
13	4.18 (1H, dd, 6.4, 13.2)	4.16 (1H, dd, 6.4, 13.2)	4.23 (1H, dd, 12.8, 6.8)	4.22 (1H, dd, 12.8, 6.4)	5.36 (m)	5.32 (m)
14	1.53 (2H, m)	1.52 (2H, m)	2.33 (2H, m)	2.33 (2H, m)	1.55 (2H, m)	2.79 (2H, t, 7.6)
15	1.26-1.37 (m)	1.26-1.37 (m)	5.36 (1H, dd, 15.5, 7.6)	5.36 (1H, dd, 15.5, 7.6)	1.20-1.40 (m)	5.32 (m)
16	1.26-1.37 (m)	1.26-1.37 (m)	5.57 (1H, m)	5.57 (1H, m)	1.20-1.40 (m)	5.32 (m)
17	1.26-1.37 (m)	1.26-1.37 (m)	2.07 (2H, m)	2.07 (2H, m)	1.20-1.40 (m)	2.10 (2H, m)
18	0.89 (3H, t, 7.5)	0.88 (3H, t, 7.5)	0.97 (3H, t, 7.5)	0.97 (3H, t, 7.5)	0.88 (3H, t, 8.8)	0.96 (3H, t, 9.6)
OMe	-	3.66 (3H, br s)	-	3.66 (3H, br s)	-	-

Table 2
^{13}C NMR (125 MHz, $CDCl_3$) data of compound 1-6.

No	1	2	3	4	5	6
1	179.0	174.5	179.1	174.5	174.9	174.9
2	34.2	34.2	33.7	34.2	34.0	34.1
3	24.5	25.0	24.5	25.0	24.8	24.4
4-6	28.8	29.1	28.9	29.1	29.0	28.7
7	29.3	29.2	29.3	29.2	28.9	28.9
8	27.5	27.8	27.6	27.8	27.1	27.6
9	132.7	132.9	132.8	133.0	128.1	127.8
10	127.8	128.0	127.8	127.9	130.2	130.9
11	125.8	125.9	125.9	126.0	29.5	29.5
12	135.6	136.1	134.9	135.3	128.1	128.1
13	72.9	73.0	72.6	72.3	130.2	128.1
14	37.2	37.5	35.2	35.4	29.2	29.5
15	25.0	25.0	123.7	124.0	27.6	131.9
16	31.7	31.9	135.2	135.3	31.5	128.6
17	22.5	22.8	20.6	20.9	22.5	20.5
18	14.0	14.0	14.2	14.4	14.0	14.2
OMe	-	51.6	-	51.6	-	-

2.5. NF-κB reporter assay

RAW264.7 cells were transiently transfected with a plasmid containing eight copies of κB elements linked to the secreted alkaline phosphatase (SEAP) gene, then the SEAP assay was performed as previously described[14]. Briefly, transfected cells were seeded in a 96-well plate at a density of 5×10^4 cells/well. After 3 h incubation at 37 ℃, cells were treated with samples and stimulated with 1 μg/mL LPS for 24 h. 100 μL of each culture supernatants were transferred to 96-well plate and heated at 65 ℃ for 10 min. followed by adding 100 μL of 2× SEAP buffer to each well and incubated for another 10 min. The reaction was initiated by adding 20 μL of 31.6 mg/mL *p*-nitrophenyl phosphate dissolved in 1× SEAP buffer and incubated at 37 ℃ for 4 h. The optical density values of solutions were measured at 405 nm with a microplate reader. Resveratrol, a potent inhibitor of NF-κB and NO production was used as positive control.

3. Results

A bioassay-guided fractionation of the methanol extract of the *C. odorata* led to the isolation of six fatty acids including (*S*)-coriolic acid (1), (*S*)-coriolic acid methyl ester (2), (*S*)-15,16-didehydrocoriolic acid (3), (*S*)-15,16-didehydrocoriolic acid methyl ester (4), linoleamide (5) and linolenamide (6). Their structures were determined by the MS and NMR spectroscopic evidences and in comparison with the reported data[15-18].

The inhibitory effects of compounds 1-6 on the NO production from LPS-stimulated RAW264 cells were evaluated by Griess method as described above. The cells were treated compounds and stimulated with 1 μg/mL LPS for 24 h and the NO levels in the culture media were measured as nitrite concentration. The result showed that compounds inhibited NO production with the IC_{50} values in the range of 5.73-47.6 μM (Table 3). The MTT assay showed that all compounds had no significant toxicity at their effective doses for the NO inhibition. Since NF-κB is considered as one of the major factors that regulate the production of NO. Therefore we examined whether the isolated compounds has inhibitory effect on NF-κB activation. The NF-κB-dependent reporter gene assay showed that compounds 1-6 inhibited NF-κB activity at concentrations comparable to the NO inhibition with IC_{50} values from 5.22 to 41.2 μM. In both assays, compound 2 exhibited the strongest activities. Interestingly, the addition of a double bond in the fatty chain decreased the inhibitory effects (in case of compounds 1-3, 2-4 and 5-6) while the methyl esterification increased the activities (in case of compounds 1-2 and 3-4).

Table 3
IC_{50} valuesa of compounds 1-6 on the NO production and NF-κB activation(μM).

Compounds	NF-κB	NO production
(*S*)-coriolic acid (1)	30.60 ± 3.66	34.10 ± 5.42
(*S*)-coriolic acid methyl ester (2)	5.22 ± 1.63	5.73 ± 1.96
(*S*)-15,16-didehydrocoriolic acid (3)	41.20 ± 4.20	47.60 ± 5.75
(*S*)-15,16-didehydrocoriolic acid methyl ester (4)	10.60 ± 1.74	12.40 ± 2.55
Linoleamide (5)	25.10 ± 3.20	24.00 ± 4.81
Linolenamide (6)	42.30 ± 5.35	36.40 ± 6.28
Resveratrol[b]	12.50 ± 1.93	10.90 ± 1.51

Data are means ± SD from three distinguished experiments, b: positive control.

4. Discussion

Fatty acids are known as self-defensive agents in organism and possess various biological activities including anti-inflammation[19-25]. Compounds 1-6 are metabolites of linoleic acid or linolenic acid which are essential fatty acids necessary for human health. It is known that NF-κB activation involves in the inflammation development and the inhibition of this transcriptional factor is considered as a therapeutic target for inflammation treatment[12]. The present study showed that the fatty acid derivatives isolated from *C. odorata* are natural inhibitors of NF-κB and NO production. Although *C. odorata* has been widely used in traditional medicine and a number of studies on the chemical and biological activity have been reported, however this is the first study on the NF-κB activity of this plant. Previous report showed that the aqueous extract of *C. odorata* significantly reduced the carrageenan-induced oedema, cotton pellet granuloma and formalin-induced oedema in rat models[26]. This anti-inflammatory effect might be related to the inhibition of NF-κB and NO production of the fatty acid components in *C. odorata*. The result of this study could explain the efficiency in medicinal usage of this plant for the anti-inflammation purpose.

Conflict of interest statement

Authors report no conflicts of interest.

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References

[1] Vo VC. *Dictionary of Vietnamese medicinal plants*. Hanoi: Medical Publisher; 1997, p. 274.

[2] Pisutthanan N, Liawruangrath B, Liawruangrath S, Baramee A, Apisariyakul A, Korth J, et al. Constituents of the essential oil from aerial parts of *Chromolaena odorata* from Thailand. *Nat Prod Res* 2006; **20**: 636-640.

[3] Biller A, Boppre M, Witte L, Hartmann T. Pyrrolizidine alkaloids in *Chromolaena odorata*. Chemical and chemoecological aspects. *Phytochem* 1994; **35**: 615-619.

[4] Ling SK, Pisar MM, Man S. Platelet-activating factor (PAF) receptor binding antagonist activity of the methanol extracts and isolated flavonoids from *Chromolaena odorata* (L.) King and Robinson. *Biol Pharm Bull* 2007; **30**: 1150-1152.

[5] Irobi ON. Activities of *Chromolaena odorata* (Compositae) leaf extract against *Pseudomonas aeruginosa* and *Streptococcus faecalis*. *J Ethnopharmacol* 1992; **37**: 81-83.

[6] Suksamrarn A, Chotipong A, Suavansri T, Boongird S, Timsuksai P, Vimuttipong S, et al. Antimycobacterial activity and cytotoxicity of flavonoids from the flowers of *Chromolaena odorata*. *Arch Pharm Res* 2004; **27**: 507-511.

[7] Amatya S, Tuladhar SM. *In vitro* antioxidant activity of extracts from *Eupatorium odoratum* L. *Res J Med Plant* 2011; **5**: 79-84.

[8] Ngono Ngane A, Ebelle ER, Ndifor F, Biyiti L, Amvam PH, Bouchet P. Antifungal activity of *Chromolaena odorata* (L.) King & Robinson(Asteraceae) of Cameroon. *Chemotherapy* 2006; **52**: 103-106.

[9] Srinivasa Rao K, Chaudhury PK, Pradhan A. Evaluation of anti-oxidant activities and total phenolic content of *Chromolaena odorata*. *Food Chem Toxicol* 2010; **48**: 729-732.

[10] Phan TT, Hughes MA, Cherry GW. Anti-oxidant effects of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine-xanthine oxidase induced damage. *Wound Repair Regen* 2001; **9**: 305-313.

[11] Anggard E. Nitric oxide: mediator, murderer, and medicine. *Lancet* 1994; **343**: 1199-1206.

[12] Kumar A, Takada Y, Boriek AM, Aggarwal BB. Nuclear factor-κB: its role in health and disease. *J Mol Med* 2004; **82**: 434-448.

[13] Schmidt HHHW, Kelm M. Determination of nitrite and nitrate by the Griess reaction. In: Feelisch M, Stamler JS. (eds.) *Methods in nitric oxide research*. New York: John Wiley & Sons Ltd; 1996, p. 491-497.

[14] Nguyen TD, Phung XB, Le TPQ, Chau VM, Hoang TH, Lee JJ. Cytotoxic prenylated flavonoids from *Morus alba*. *Fitoterapia* 2010; **81**: 1224-1227.

[15] Yadav JS, Deshpande PK, Sharma GVM. Stereoselective synthesis of *(S)*-13-hydroxy octadeca-(9*Z*,11*E*)-di- and (9*Z*,11*E*,15*Z*)-trienoic acids: selfdefensive substances against rice blast disease. *Tetrahedron* 1992; **48**: 4465-4474.

[16] Kuklev DV, Christie WW, Durand T, Rossi JC, Vidal JP, Kasyanov SP, et al. Synthesis of keto- and hydroxydienoic compounds from linoleic acid. *Chem Phys Lipids* 1997; **85**: 125-134.

[17] Xua MZ, Lee WS, Kim MJ, Park DS, Yu H, Tiac GR, et al. Acyl-CoA: cholesterol acyltransferase inhibitory activities of fatty acid amides isolated from *Mylabris phalerate* Pallas. Bioorg Med. *Chem Lett* 2004; **14**: 4277-4280.

[18] Yang NY, Li K, Yang YF, Li YH. Aromatase inhibitory fatty acid derivatives from the pollen of *Brassica campestris* L. var. oleifera DC. *J Asian Nat Prod Res* 2009; **11**: 132-137.

[19] Gautam R, Jachak SM. Recent developments in anti-inflammatory natural products. *Med Res Rev* 2009; **29**: 767-820.

[20] Vijayalakshmi A, Ravichandiran V, Velraj M, Hemalatha S, Sudharani G, Jayakumari S. Anti-anaphylactic and anti-inflammatory activities of a bioactive alkaloid from the root bark of *Plumeria acutifolia* Poir. *Asian Pac J Trop Biomed* 2011; **1**(5): 401-405.

[21] Kumar S, Kumar V, Prakash OM. Pharmacognostic study and anti-inflammatory activity of *Callistemon lanceolatus* leaf. *Asian Pac J Trop Biomed* 2011; **1**(3): 177-181.

[22] Simopoulos AP. Essential fatty acids in health and chronic disease. *Am Clin Nutr* 1999; **70**: 560S-569S.

[23] Mohamed Saleem TK, Azeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf exacts of *Gendarussa vulgaris* Nees. *Asian Pac J Trop Biomed* 2011; **1**(2): 147-149.

[24] Okokon JE, Udoh AE, Frank SG, Amazu LU. Anti-inflammatory and analgesic activities of *Melanthera scandens*. *Asian Pac J Trop Biomed* 2012 Jan 28; **2**(2): 144-148. Epub 2011 Sep 7.

[25] Kumar S, Kumar V, Prakash OM. Pharmacognostic study and anti-inflammatory activity of Callistemon lanceolatus leaf. *Asian Pac J Trop Biomed* 2011; **1**(3): 177-181.

[26] Owoyele VB, Adediji JO, Soladoye AO. Anti-inflammatory activity of aqueous leaf extract of *Chromolaena odorata*. *Inflammopharmacology* 2005; **13**(5-6): 479-484.