Anti-inflammatory effect of methanolic extract of *Conyzacanadensis* in lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophage cells

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Abstract: The aim of this study was to investigate the potential anti-inflammatory effect of *Conyzacanadeusis* methanol extract (CME) using a cell model of RAW264.7 murine macrophage cell stimulated with lipopolysaccharide (LPS)(1μg/ml). Co-treatment with different concentrations (10, 50 and 100μg/ml) of CME was concentration-dependently reduced the LPS-induced generation of prostaglandin E2 (PGE2), nitric oxide (NO) tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6. In addition, CME also reduced the mRNA expressions of cyclooxygenase-2 (COX-2), inducible nitric oxide (iNOS), TNF-α, IL-1β and IL-6 in LPS-stimulated RAW264.7 cells. These results suggested that CME showed an anti-inflammatory activity through reduced the mRNA expression of COX-2, iNOS, TNF-α, IL-1β and IL-6 and also decreased the productions of PGE2, NO, TNF-α, IL-1β and IL-6in LPS-stimulated RAW264.7 cells.

Keywords: *Conyzacanadensis*, anti-inflammation, RAW264.7 cells.

INTRODUCTION

Inflammation is a normal physiologic protective reaction of the body through activates the immune system to against pathogen-induced infection and harmful stimuli-induced tissue injury (Hagemann *et al*., 2007). However, inflammation also is a "double-edged sword". Chronic inflammation was associated with pathological process of several diseased conditions or disorders such as asthma, relueumatoid, arthritis, atherosclerosis, Alzheimer's disease and cancer (Krishnamoorthy and Honn, 2006). The high levels of some pro-inflammatory mediators, such as pro-inflammatory cytokines (e.g. interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α), nitric oxide (NO), prostaglandin E2 (PGE_2), as well as inflammation-related enzymes including cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was play a very important role in the process of these chronic inflammation-related disease (Ben-Baruch, 2006; Germano *et al*., 2008). Reduction of these pro-inflammatory mediators is a target to treat chronic inflammatory disease. Generally, the non-steroidal anti-inflammatory drugs (NSAIDs) are a type of basic and common compound for clinical anti-inflammatory treatment. However, prolonged administration of NSAIDs was associated with severe gastrointestinal and cardiovascular side-effects (Jones *et al*., 2008). Base on these results, plant-derived nature compounds have been focused on the treatment of various inflammatory disease due to high efficaciousness and no side-effects (Calixto *et al*., 2004).

Conyzacanadensis (L.) Cronq. is a annual plant nativeto North America and now distributed globally(Weaver, 2001). It also has been recognized as an invasive plant in China (Ling *et al*., 1985). However, the whole plants of *Conyzacanadensis* have been used medicinallyin China to treat edema, hematuria, hepatitis and cholecystitis (Ling *et al*., 1985). Other studies also reported that *Conyzacanadensis* exhibited antimicrobial (Shakirullah *et al*., 2001), antifungal (Queiroz *et al*., 2012), antioxidant (Olas *et al*., 2006; Saluk-Juszczak *et al*., 2010), antiaggregatory (Olas *et al*., 2006; Saluk-Juszczak *et al*., 2007), anti-melanoma (Yan *et al*., 2010), anti-inflammatory activities (Lenfeld *et al*., 1986) and catecholamine secretion inhibitor activity (Ding *et al*., 2010).

This study was designed to investigate the potential anti-inflammatory activity of CME on LPS-stimulated RAW264.7 cells and also to elucidate the mechanisms underlying the anti-inflammatory effect of CME by evaluating the productions of several pro-inflammatory mediators, including COX-2, iNOS, TNF-α, IL-1β and IL-6.

MATERIAL AND METHODS

Plant extracts preparation

Fresh *Conyzacanadensis* were purchased from a local

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market in Yangzhou, China in May 2012. The fresh epigean part of *Conyzacanadensis* were freeze dried and then ground to a fine powder. A twelve-fold volume of methanol (80%, v/v) was added to the powdered samples and extracted third by stirring overnight. *Conyzacanadensis* methanolic extracts (CME) were concentrated by heat evaporation, freeze-drying and stored at 4°C for further study.

Cell culture

RAW 264.7 murine macrophage cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were maintained in RPMI 1640 medium supplemented 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified incubator (model 3110; Forma Scientific, Inc., Marietta, OH, USA) with 5% CO_2 at 37°C.

Cell viability assay

Cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazoliumbromide (MTT) assay. Cells were seeded on 96-well cell culture plates at a density of 5×10^4 cells/well. Following 24h incubation, the cells were co-incubated with CME (10, 50 and 100μg/ml) and LPS (1μg/ml) for 24h. Following treatment, 100μl MTT reagent (0.5mg/ml) was added to each well, and then the cells was incubated at 37°C to allow the MTT to be metabolized. After 4h, the medium was removed, and cells were resuspended in formazan with 100μl DMSO. The absorbance of the samples was measured using a micro plate reader (model 680, Bio-Rad, Hercules, CA, USA) at 540 nm.

Assay for prostaglandin E2 (PGE2) production

RAW264.7 cells grown in 6-cell culture plates were co-incubated with CME (10, 50 and 100μg/ml) and LPS (1 μg/ml) for 24 h. Following treatment, aliquots of samples (100μl/well) were collected from the experimental medium, and the PGE_2 productions were measured using a commercial ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocols.

Assay for nitrite oxide (NO) production

The NO production in the cell culture medium was determined by Griess reaction using a commercial nitric oxide assay kit (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. First, the culture medium was collected from each group at the end of test. A total 100μl of culture medium was mixed with an equal volume of Griess reagent containing1% sulfanilamide, 0.1% naphthylethylenediaminedihydrochloride and 2% phosphoric acid and then kept them at room temperature for 10min. The absorbance was measured at 540nm using a micro plate reader (model 680). The amount of nitric was calculated according toa sodium nitrate standard curve.

Assay for pro-inflammation cytokines (TNF-α, IL-1β and IL-6) levels

TNF-α, IL-1β and IL-6 productions were measured with an ELISA assay.RAW264.7 cells grown in 6-cell culture plates were co-incubated with CME (10, 50 and100μg/ml) and LPS (1μg/ml) for 24h. At the end of the experiment, aliquots of samples (100μl/well) were collected from the experimental medium, and the cytokines (TNF-α, IL-1β and IL-6) productions were measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocols.

Reverse transcription polymerase chain reaction (RT-PCR) assay

mRNA expression of COX-2, iNOS, TNF-α, IL-1β and IL-6was measured with an RT-PCR assay. Total cellular RNA was isolated with Trizol reagent (Invitrogen, Carlsbad, CA, USA) and centrifuged at 12,000 ×g for 15 min at 25°C following the addition of chloroform. Isopropanol was added to the supernatant at a 1:1 ratio and the RNA was pelleted by centrifugation (12,000 ×g for 15 min at 4°C). After washing with 70% ethanol, the RNA was solubilized in diethyl pyrocarbonate (DEPC)-treated RNase-free double distilled water and quantified by measuring the absorbance using a UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan) at 260 nm. Equal amounts of RNA (1 μg) were reverse transcribed in a AccuPower PCR PreMix (Bioneer, Daejeon, South Korea) containing 1 × reverse transcriptase buffer, dNTPs (1mM), oligodT_{18} primers (500ng), MMLV reverse transcriptase(140 U), and RNase inhibitor(40 U) for 45 min at 42°C. PCR was then carried out in an automatic PCR thermocycler (Bioneer, Daejeon, South Korea) for 20 cycles (94°C for 60 s, 60°C for 60 s, and 72°C for 60 s) followed by an 5-min extension at 72°C. The PCR products were separated in 2% agarose gels and visualized by EtBr staining. β-actin was used for normalization.

STATISTICAL ANALYSIS

Data are presented as the mean ± standard deviation (SD). Differences between the mean values for individual groups were assessed by a one-way ANOVA with Duncan's multiple range tests. P-values <0.05 was considered statistically significant. The SAS v9.1 statistical software package (SAS Institute Inc., Cary, NC, USA) was used to perform the statistical analysis.

RESULTS

Effect of CME on cell viability in RAW264.7cells

To investigate the CME-induced cytotoxicity, RAW264.7 cells were co-incubated with various concentrations of CME (10, 50 and 100μg/ml) with or without LPS (1μg/ml) for 24 h and the cell viability was evaluated with MTT assays. As shown in fig. 1, CME did not exhibit

Table 1: Effects of *Conyzacanadensis* methanolic extracts (CME) on LPS (1μg/ml)-induced prostaglandin E_2 (PGE_2) and nitric oxide (NO) and generation.

Treatment	PGE_2 (pg/ml)	Nitric oxide (μM)
Control	6.2±0.6[d]	11.9±2.1[d*]
LPS (1 μg/ml)	45.0±6.1[a]	57.7±10.6[a]
LPS + CME (10μg /ml)	32.3±1.5[b]	45.7±5.7[b]
LPS+CME (50μg /ml)	25.9±1.2[c]	36.5±4.5[bc]
LPS+CME (100μg /ml)	22.6±1.1[c]	30.6±3.8[c]

[*] Data are representative of three independent experiments as mean ± SD. [a~e] Mean values with different letters on the bars are significantly different (P<0.05) according to Duncan's multiple range test.

Table 2: Effects of *Conyzacanadensis* methanolic extracts (CME) on LPS (1μg/ml)-induced pro-inflammatory cytokines formation.

Treatment	Concentration (pg/ml)		
	TNF-α	IL-1β	IL-6
Control	15.4±5.0[d*]	19.9±5.5[e]	26.9±5.9[e]
LPS (1μg /ml)	1493.2±9.6[a]	790.7±10.1[a]	1147.3±51.5[a]
LPS+ CME (10 μg/ml)	1470.3±11.5[a]	765.7±14.6[b]	1017.0±62.4[b]
LPS+CME (50 μg/ml)	1225.5±50.7[b]	612.5±11.7[c]	813.6±49.9[c]
LPS+CME (100μg/ml)	1127.3±85.6[c]	536.0±10.2[d]	711.9±43.7[d]

[*]Data are representative of three independent experiments as mean ± SD. [a~e] Mean values with different letters on the bars are significantly different (P<0.05) according to Duncan's multiple range test. TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1β; IL-6: interleukin-6.

significantly cytotoxicity on RWA264.7 cells (the cell viability are both >100%). In addition, LPS (1μg/ml) significantly decreased the cell viability (P<0.05). However, co-incubated with various of CME was able to increased the cell viability ina concentration-dependent manner.

Effect of CME on PGE2levels in LPS-stimulated RAW264.7 cells

PGE2is a good indicator to evaluate the degree of inflammation. As shown in table1, LPS significantly increased the PGE2 levels (to 45.0±6.1 pg/ml) than that of normal cells (6.2±0.6pg/ml). CME was able to concentration-dependently decreased the PGE2 levels co-incubated with LPS in RAW 264.7 cells.

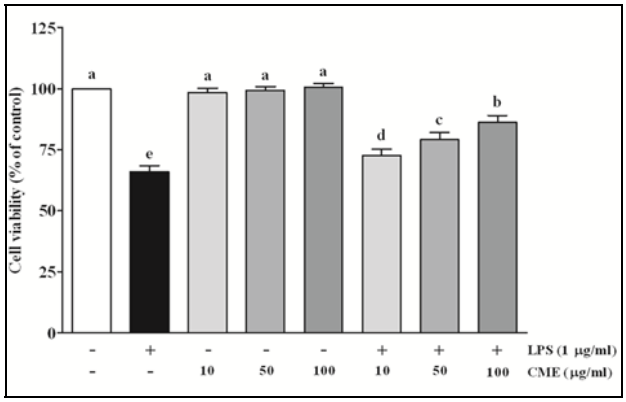

Fig. 1: Effects of *Conyzacanadensis* methanolic extracts (CME) on cell viability in RAW264.7 cells. Data are representative of three independent experiments as mean ± SD. [a~e] Mean values with different letters on the bars are significantly different (P<0.05) according to Duncan's multiple range test.

Effect of CME on NO levels in LPS-stimulated RAW264.7 cells

It isknown that NO also is a pro-inflammatory mediator and plays an important role in the process of inflammation. To evaluate the effect of CME on NO generation, the amount of nitrite, a stable final product of NO were determined using Griessreagent. As shown in table 1, LPS (1μg/ml) significantly increased the NO levels in RAW264.7 cells (P<0.05). However, co-incubated with CME (10, 50 and 100μg/ml) was able to concentration-dependently reduced the LPS-stimulated NO productions in RAW264.7 cells.

Effect of CME on pro-inflammatory cytokine levels in LPS-stimulated RAW264.7 cells

Table 2 shows the effect of CME on the pro-inflammatory levels of TNF-α, IL-1β and IL-6 in LPS-stimulated RAW264.7 cells. LPS (1μg/ml) significantly (P<0.05) increased the TNF-α, IL-1β and IL-6 levels (to 1493.2± 9.6,790.7±10.1 and 1147.3±51.5pg/ml, respectively) than those of normal cells (15.4±5.0,19.9±5.5 and 26.9±5.9 pg/ml, respectively), respectively. In the presence of LPS, CME showed a concentration-dependently reduction activity of TNF-α, IL-1β and IL-6 than those of control cells (RAW264.7 cells treated with LPS alone).

Effect of CME on mRNA expressions of COX-2, iNOS, TNF-α, IL-1β andIL-6 in LPS-stimulated RAW264.7 cells

To further investigate the anti-inflammatory effect of CME in LPS-stimulated RAW264.7 cells. mRNA expression of iNOS, COX-2,TNF-α,IL-1β andIL-6 were measured with an RT-PCR assay. LPS (1μg/ml) significantly ($P<0.05$) increased the mRNA expression of COX-2, iNOS, TNF-α, IL-1β andIL-6 compared with that in the normal cells (fig. 2 and 3). Co-incubated with LPS and CME (10, 50 and 100μg/ml) for 24h significantly reduced the mRNA expression of COX-2, iNOS, TNF-α, IL-1β and IL-6 than those of only LPS-stimulated cells.

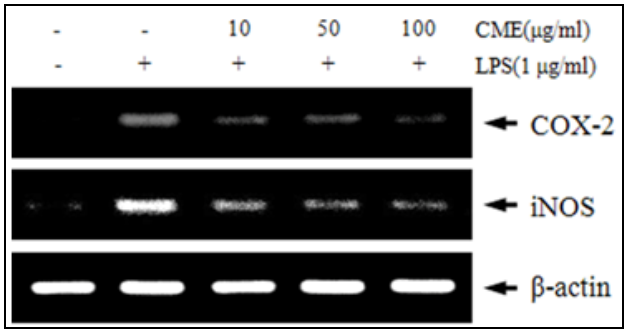

Fig. 2: Effects of *Conyzacanadensis* methanolic extracts (CME) on mRNA expressions of iNOS and COX-2 in LPS (1μg/ml)-stimulated RAW264.7 cell.

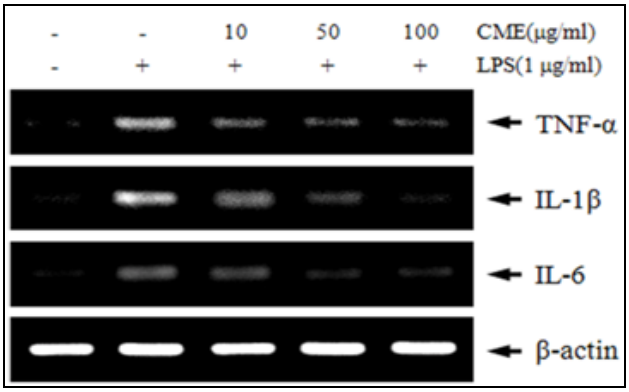

Fig. 3: Effects of *Conyzacanadensis* methanolic extracts (CME) on mRNA expressions of TNF-α, IL-1βand IL-6 in LPS (1μg/ml)-stimulated RAW264.7 cells.

DISCUSSION

Inflammation is a complex biological reaction and associated with the increasing of server pro-inflammatory factors, such as pro-inflammatory cytokines, NO and PGE_2. Chronic inflammation was also resulted in severe disease, including atherosclerosis, Alzheimer's disease and cancer (Krishnamoorthy and Honn, 2006). In this study, we observed that CME was able to reduce the LPS-induced generation of TNF-α, IL-1β and IL-6 as well as their mRNA expression in LPS-stimulated RAW264.7 cells. In addition, we also demonstrated that CME inhibits NO and PGE2 productions through modulating the mRNA expression of COX-2 and iNOS in LPS-treated cells.

PGE_2 is a product generated from arachidonic acid through COX-2 catalytic reaction and also as an important mediator involved in process of chronic inflammation related disease (Patrignani *et al*., 2005; Shoji *et al*., 2007). COX-2 is animportantenzyme associated with the development of inflammation and cancer, and it also is apivotal target for anti-inflammatory treatment (O'banion, 1999; Patrignani *et al*., 2005). Reduction of COX-2/PGE_2pathway has been proposed to be a useful approach for treating various inflammatory diseases as well as influencing the colorectal tumorigenesis (Leone *et al*., 2007; Greenhough *et al*., 2009). We observed that CME (10, 50, and 100μg/ml) significantly reduced the LPS-stimulated PGE_2 generation, and also decreased the mRNA level of COX-2 in LPS-treated RAW264.7 cells, suggesting possible beneficial effects of CME by modulating the activation of macrophage cells and attenuates subsequent inflammatory disease.

NO, a "Molecule of the Year" proclaimed by Science journal in 1992 (Culotta and Koshland, 1992), is an important cellular signaling molecule generated from L-argininеionsand also andalso as a "double-edged sword "involved in many physiological and pathological processes (Ignarro, 1991). It is well known that normal levels of NO exhibited the anti-tumor, anti-virus and anti-inflammatory activities (MacMicking *et al*., 1997). However, high levels of NO produced fromover activation of iNOS was resulted in the chronic inflammation-related diseases (Cirino *et al*., 2006). Base on these reasons, decreased the overproduction of NO has become a new therapeutic target for anti-inflammatory treatment (Cirino *et al*., 2006; Kawanishi *et al*., 2006). In this study, LPS significantly increases the NO generation in RAW264.7 cells. However, co-incubated with different concentrations (10, 50 and 100μg/ml) of CME was able to reduce the LPS-stimulated NO generation, as well as the mRNA expression of iNOS in RAW264.7 cells.

In addition to the $PGE_2$and NO, pro-inflammatory cytokines form the activated macrophages also plays a main role during the pathological process of chronic inflammation-related disease. As an important pro-inflammatory cytokine, TNF-α is able to increase the generation of some other inflammation-related cytokines, such as IL-6 IL-1βand interferon-γ (Butler *et al*., 1997). Elevated levels of IL-6, IL-1βand TNF-α were also observed in chronic inflammatory disease (Butler *et al*., 1997). Suppression of TNF-α, IL-6 and IL-1βwere attenuated the inflammatory reaction in human diseases (Möller and Villiger, 2006). Therefore, inhibition of the activated pro-inflammatory cytokines is a key mechanism in the control of the inflammation (Feghali and Wright,

1997). In this study, we found CME concentration-dependently inhibits the productions of TNF-α, IL-1β and IL-6 in LPS-stimulated RAW264.7 cells. In addition, CME also significantly reduced the mRNA expression of TNF-α, IL-1β and IL-6 compared with those in the LPS-stimulated RAW264.7 cells. These results indicate that the anti-inflammatory activity of CME in LPS-treated RAW264.7 cells is through reduction of the activities of TNF-α, IL-1β and IL-6.

CONCLUSION

In this study, we reported that CME showed an anti-inflammatory activity on LPS-stimulated RAW264.7 cells. The anti-inflammatory activity of CME may be due to decrease thepro-inflammatory factors (PGE_2andNO) generation through modulating the mRNA expression of COX-2 and iNOS and also reduced the activation of TNF-α, IL-1β and IL-6 in LPS-stimulated RAW264.7 cells. These results have also revealed the promising application of *Conyzacanadensis* in treatment of chronic inflammatory disease.

REFERENCES

Ben-Baruch A (2006). Inflammation-associated immune suppression in cancer: The roles played by cytokines, chemokines and additional mediators. *Semin. Cancer Biol.*, **16**: 38-52.

Butler DM, Malfait AM, Mason LJ, Warden PJ, Kollias G, Maini RN, Feldmann M and Brennan FM (1997). DBA/1 mice expressing the human TNF-alpha transgene develop a severe, erosive arthritis: Characterization of the cytokine cascade and cellular composition. *J. Immunol.*, **159**: 2867-2876.

Calixto JB, Campos MM, Otuki MF and Santos AR (2004). Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta. Med.*, **70**: 93-103.

Cirino G, Distrutti E and Wallace JL (2006). Nitric oxide and inflammation. *Current Drug Targets*, **5**: 115-119.

Culotta E and Koshland Jr DE (1992). NO news is good news. *Science*, **258**: 1862-1865.

Ding Y, Su Y, Guo H, Yang F, Mao H, Gao X, Zhu Z and Tu G (2010). Phenylpropanoyl esters from horseweed (*Conyza canadensis*) and their inhibitory effects on catecholamine secretion. *J. Nat. Prod.*, **73**: 270-274.

Feghali CA and Wright TM (1997). Cytokines in acute and chronic inflammation. *Front. Biosci.*, **2**: 12-26.

Germano G, Allavena P and Mantovani A (2008). Cytokines as a key component of cancer-related inflammation. *Cytokine*, **43**: 374-379.

Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C and Kaidi A (2009). The COX-2/PGE2 pathway: Key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis*, **30**: 377-386.

Hagemann T, Balkwill F and Lawrence T (2007). Inflammation and cancer: A double-edged sword. *Cancer cell*., **12**: 300-301.

Ignarro LJ (1991). Signal transduction mechanisms involving nitric oxide. *Biochem. Pharmacol.*, **41**: 485-490.

Jones R, Rubin G, Berenbaum F and Scheiman J (2008). Gastrointestinal and cardiovascular risks of nonsteroidal anti-inflammatory drugs. *Am. J. Med.*, **121**: 464-474.

Kawanishi S, Hiraku Y, Pinlaor S and Ma N (2006). Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biol. Chem.*, **387**: 365-372.

Krishnamoorthy S and Honn KV (2006). Inflammation and disease progression. *Cancer Metastasis Rev.*, **25**: 481-491.

Lenfeld J, Motl O and Trka A (1986). Anti-inflammatory activity of extracts from *Conyza canadensis*. *Pharmazie*, **41**: 268-269.

Leone S, Ottani A and Bertolini A (2007). Dual acting anti-inflammatory drugs. *Curr. Top Med. Chem.*, **7**: 265-275.

LingY, Chen Y and Shih C (1985). Flora Reipublicae Popularis Sinicae. Tomus 74; Science Press: Beijing, China. pp.348-350.

MacMicking J, Xie QW and Nathan C (1997). Nitric oxide and macrophage function. *Annu. Rev. Immunol*., **15**: 323-350.

Möller B and Villiger PM (2006). Inhibition of IL-1, IL-6, and TNF-α in immune-mediated inflammatory diseases. *Springer Semin. Immunopathol.*, **27**: 391-408.

O'banion M (1999). Cyclooxygenase-2: Molecular biology, pharmacology, and neurobiology. *Crit. Rev. Neurobiol.*, **13**: 45-82.

Olas B, Saluk-Juszczak J, Pawlaczyk I, Nowak P, Kolodziejczyk J, Gancarz R and Wachowicz B (2006). Antioxidant and antiaggregatory effects of an extract from *Conyza canadensis* on blood platelets *in vitro*. *Platelets*, **17**: 354-360.

Patrignani P, Tacconelli S, Sciulli MG and Capone ML (2005). New insights into COX-2 biology and inhibition. *Brain Res. Brain Res. Rev.*, **48**: 352-359.

Queiroz SC, Cantrell CL, Duke SO, Wedge DE, Nandula VK, Moraes RM and Cerdeira AL (2012). Bioassay-directed isolation and identification of phytotoxic and fungitoxic acetylenes from *Conyza canadensis*. *J. Agric. Food Chem.*, **60**: 5893-5898.

Saluk-Juszczak J, Olas B, Nowak P, Wachowicz B, Bald E, Głowacki R, Pawlaczyk I and Gancarz R (2010). Extract from *Conyza canadensis* as a modulator of plasma protein oxidation induced by peroxynitrite *in vitro*. *Cent. Eur. J. Biol.*, **5**: 800-807.

Saluk-Juszczak J, Olas B, Pawlaczyk I, Gancarz R and Wachowicz B (2007). Effects of the extract from

Conyza canadensis on human blood platelet aggregation. *Gen. Physiol. Biophys.*, **26**: 150-152.

Shakirullah M, Ahmad H, Shah MR, Ahmad I, Ishaq M, Khan N, Badshah A and Khan I (2011). Antimicrobial activities of Conyzolide and Conyzoflavone from *Conyza canadensis*. *J. Enzyme Inhib. Med. Chem.*, **26**: 468-471.

Shoji M, Tanabe N, Mitsui N, Suzuki N, Takeichi O, Katono T, Morozumi A and Maeno M (2007). Lipopolysaccharide enhances the production of nicotine-induced prostaglandin E2 by an increase in cyclooxygenase-2 expression in osteoblasts. *Acta. Biochim. Biophys. Sin. (Shanghai)*, **39**: 163-172.

Weaver SE (2001). The biology of Canadian weeds. 115. *Conyza canadensis*. *Can. J. Plant Sci.*, **81**: 867-875.

Yan MM, Li TY, Zhao DQ, Shao S and Bi SN (2010). A new derivative of triterpene with anti-melanoma B16 activity from *Conyza canadensis*. *Chinese Chem. Lett.*, **21**: 834-837.