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Functional food components of *Antigonon leptopus* tea

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Abstract

The tubers and flowers of *Antigonon leptopus*, belonging to the family Polygonaceae, are consumed as food in several parts of the world. The aerial portion, including flowers, is used in the preparation of tea used as a cold remedy. This prompted us to investigate its functional food qualities and found that the methanol extract of the aerial parts of *A. leptopus*, inhibited lipid peroxidation (LPO) by 89% and cyclooxygenase enzymes, COX-1 and COX-2 by 50.4% and 72.5%, respectively, at 250 μg/ml. Purification of the methanolic extract yielded *n*-hentriacontane (**1**), ferulic acid (**2**), 4-hydroxycinnamic acid (**3**), quercetin-3-rhamnoside (**4**), and kaempherol-3-glucoside (**5**) along with β-sitosterol, β-sitosterol-glucoside and d-mannitol. Compounds **3**, **4** and **5** inhibited LPO by 19.5%, 41.0%, and 60.5%, respectively, at 5 μg/ml. Similarly, compounds **3**, **4** and **5** inhibited COX-1 enzyme by 64.7%, 16.9%, and 38.5% and COX-2 enzyme by 87.4%, 88.8%, and 90.2%, respectively, at 25 μg/ml. Compounds **3**, **4** and **5** showed 50% inhibition (IC_{50}) at 17.4%, 68.9% and 36.3 μg/ml, against COX-1 and 8.57%, 7.86% and 6.78 μg/ml for COX-2, respectively. This is the first report of the isolation of compounds **2**–**5** from *A. leptopus* and their LPO and COX inhibitory activities.

Keywords: Antioxidant; Antiinflammatory; Cyclooxygenase enzyme; Flavonoids; Lipid peroxidation; Polyphenol

1. Introduction

Many plants used as ethnic foods are getting consumer's attention and *Antigonon leptopus*, known as coral vine and queen's wreath, is one of the examples. It is native to Mexico and commonly found in tropical Asia, Africa, Caribbean and the Americas (Raju, Raju, Victor, & Naidu, 2001). It is a climbing plant, that flourishes throughout the lowlands, beside streams and gullies as well as on dry and sandy heaps along the coast. The vines of this plant regularly display a pleasing veil of green, followed by sprays of pink flowers. Although *A. leptopus* plants appear to spring spontaneously from bare earth, they are in fact perennial plants with edible tubers similar to the size of small eggs (Henry & Harris, 2002). In Thailand, the fried flour-coated leaves and flowers of *A. leptopus* are served with noodles. The flowers are also used in omelets (Charmaine, 1998). A hot tea prepared from the aerial portion of this plant, is used as a treatment for cough and throat constriction in Jamaica and considered as one of the important medicinal plants in their folk-medicine (Mitchell & Ahmad, 2006). Previous studies have shown that *A. leptopus* plant extracts, exhibited potential anti-thrombin and anti-diabetic activities (Chistokhodova et al., 2002; Lans, 2006). Our interest was to investigate the impact of *A. leptopus* extracts and isolated compounds on lipid peroxidation (LPO) and cyclooxygenase (COX) enzymes, in order to examine its anecdotal medicinal claims.

Increasing evidence from epidemiological and biological studies has shown that reactive oxygen species (ROS) are

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involved in a variety of physiological and pathological processes (Halliwell & Gutteridge, 1995; Halliwell, Aeschbach, Loliger, & Aruoma, 1995). For example, ROS mediated lipid peroxidation (LPO) and DNA damage are associated with several chronic health problems, such as cancer, ageing and atherosclerosis (Marnett, 2002). Plant and food derived antioxidants are implicated in the prevention of cancer and aging by destroying oxidative species that initiate carcinogenesis through oxidative damage of DNA (Williams, Dashwood, Hendricks, & Bailey, 1989). Therefore, inhibition of LPO by supplementation of functional food antioxidants has merit for prevention of diseases.

Cyclooxygenase enzymes (COX) catalyze the production of eicosanoid lipid products from arachidonic acid and play an important role in the inflammatory processes and tumour initiation (Bome, 1995). A constitutive COX-1 enzyme present in most cells and tissues, an inducible COX-2 enzyme observed in many cells in response to pro-inflammatory cytokines and a COX-1 variant protein named COX-3, which is sensitive to acetaminophen, are three isoforms of COX enzyme. It is well known that COX-2 plays a role in the inflammation process, while inhibition of COX-1 is responsible for the adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs). Therefore, drugs that selectively inhibit COX-2 enzymes should have the beneficial anti-inflammatory activity of traditional NSAIDs without the toxicity. Consequently, COX-2 selective inhibitors drew much attention as potential candidates of anti-inflammatory agents with reduced side effects (Smith, De Witt, & Garavito, 2000).

In this paper, we report the LPO and COX inhibitory activities of extracts and isolated compounds from *A. leptopus*. The isolation of compounds **2**–**5** from this plant and the biological activities are reported here for the first time.

2. Materials and methods

2.1. General methods

Melting points were determined on Bristoline and V-Scientific melting point apparatus (MP-1) and are uncorrected. The NMR (^{1}H and ^{13}C) experiments were recorded on Varian INOVA (300 MHz) and VRX (500 MHz) instruments. ^{13}C NMR spectra were recorded at 75 and 125 MHz, respectively. The chemical shifts were measured in $CDCl_3$, DMSO-d_6 or MeOH-d_4 and are expressed in δ (ppm). Mass spectra were recorded at the Michigan State University Mass Spectrometry Facility using a JEOL HX-110 double focusing mass spectrometer (Peabody, MA). Merck silica gel (60 mesh size, 35–70 μm) was used for medium pressure liquid chromatography (MPLC). For preparative TLC separation, 500 μm silica gel plates (Analtech, Inc., Newark, DE) were used. TLC plates were viewed under UV light at 254 and 366 nm or sprayed with 10% sulfuric acid solution in methanol. ACS grade solvents were used for isolation and purification. The COX-1 enzyme was prepared from ram seminal vesicles purchased from Oxford Biomedical Research, Inc. (Oxford, MI). The COX-2 enzyme was prepared from insect cells, cloned with human PGHS-2 enzyme. Aspirin, Celebrex, and Vioxx® were used as positive controls in COX enzyme inhibitory assay. The lipid, phospholipid 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (SLPC), was purchased from Avanti Polar Lipids (Alabaster, AL). The fluorescent probe, 3-[*p*-(6-phenyl)-1, 3, 5-hexatrienyl]-phenylpropionic acid was purchased from Molecular Probes (Eugene, OR). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ), were used as positive controls in lipid peroxidation assay and purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Concentrations are expressed in μg/ml.

2.2. Plant material

Aerial parts of *A. leptopus* were collected in Jamaica during 2005 and authenticated by Mr. Patrick Lewis at University of the West Indies, Mona, Kingston, Jamaica. A voucher specimen (UWI 35294) has been deposited at the herbarium of the University of the West Indies, Mona, Kingston, Jamaica.

2.3. Extraction and Isolation

Aerial parts of *A. leptopus* (0.5 kg) were extracted sequentially with hexane (2 L × 3), ethyl acetate (2 L × 3) and methanol (2 L × 3). After removal of solvents at reduced pressure (80 mbar and 40 °C), the yields of hexane, ethyl acetate and methanol extracts were 3.8, 6.1 and 25 g, respectively. The methanol extract (25 g) was subjected to silica gel medium pressure liquid chromatography (MPLC) using chloroform and methanol mixtures of increasing polarity to yield five fractions (A–E). The fractions were collected in 250 ml aliquots.

Fraction A (1.7 g, $CHCl_3$:MeOH, 95:5) was subjected to silica gel MPLC column using mixtures of hexane and ethyl acetate. A total of 20 fractions in 50 ml aliquots were collected and then combined into two major fractions, A_1 (0.1 g, hexane:EtOAc, 90:10) and A_2 (1.2 g, hexane:EtOAc, 80:20) based on their TLC profiles. The fraction A_1 showed as a single spot by TLC (using hexane and ethyl acetate: 9:1 as mobile phase) and then recrystallized from a mixture of hexane and chloroform, to obtain β-sitosterol (8 mg). Concentration of fraction A_2 yielded a yellow solid and gave a single spot on TLC. Recrystallization of this solid from chloroform and methanol yielded compound **1** (500 mg) as white flakes. Compound **1** was identified as *n*-hentriacontane by comparing the physical and spectral data with those reported in the literature (Hasan & Burdi, 1991).

Fraction B (2.6 g, $CHCl_3$/MeOH, 90:10) was purified further by using MPLC silica gel column and eluted with hexane and ethyl acetate as the mobile phase. Each fraction was collected in 50 ml aliquots and the fractions were

pooled into two major fractions, B_1 (0.8 g, hexane:EtOAc, 80:20) and B_2 (0.5 g, hexane:EtOAc, 70:30) based on their TLC profiles. Fraction B_2 was purified further by MPLC silica gel column and eluted with hexane and ethyl acetate as the solvent system. Fractions were combined based on their TLC profiles and were concentrated at reduced pressure. The resulted solid was recrystallized from chloroform and acetone to obtain compound **2** (26 mg) and identified as 3-methoxy-4-hydroxycinnamic acid (ferulic acid) by comparison of its spectral data with those of published data (Wu, Leu, & Chan, 1998).

Fraction C (4.3 g, $CHCl_3$:MeOH, 85:15) showed two spots by TLC and was purified further by MPLC silica gel column using mixtures of chloroform and methanol as mobile phases. A total of 50 fractions in 25 ml portions were collected. Based on their TLC, profiled fractions were pooled into two major fractions, C_1 (0.9 g, $CHCl_3$:MeOH, 90:10) and C_2 (0.7 g, $CHCl_3$:MeOH, 80:20). Fraction C_1 was concentrated under reduced pressure and recrystallized from methanol and acetone to obtain compound **3** (13 mg). Compound **3** was identified as 4-hydroxycinnamic acid by comparison of its spectral data with data reported in the literature (Owen et al., 2000). Fraction C_2 ($CHCl_3$:MeOH, 80:20), showed a single spot on TLC, and was recrystallized (chloroform and methanol, 9:1, v/v) to yield β-sitosterol glucoside (10 mg) (Wu, Chan, & Leu, 2000).

Fraction D (6.8 g, $CHCl_3$:MeOH, 80:20) was subjected to MPLC silica gel column chromatography and was eluted with chloroform and methanol as the solvent system. A total of 50 fractions in 50 ml aliquots were collected. Fractions were combined based on TLC profiles and the pooled up fractions gave D_1 (1.5 g, $CHCl_3$:MeOH, 90:10) and D_2 (3.1 g, $CHCl_3$:MeOH, 80:20), which were purified further by MPLC column chromatography using chloroform and methanol as eluents. Fractions that were similar on TLC were combined and subjected to silica gel preparative TLC using chloroform:methanol:acetic acid (8:2:0.1 v/v/v) as the mobile phase. Two bands (I and II) were collected and visualized under 254 and 366 nm. Each time 50 mg of the mixture was subjected to preparative silica gel TLC and the two bands were collected individually. Combined band I fractions were extracted with chloroform and concentrated at reduced pressure and yielded a yellow gummy residue which was recrystallized (chloroform:acetone, 9:1, v/v) to give compound **4**(500 mg). Similarly, band II was extracted with chloroform, concentrated under reduced pressure and recrystallized from chloroform and acetone to yield a yellow solid, compound **5** (7 mg). Compound **4** was identified as quercitin-3-rhamnoside and compound **5** as kaempherol-3-glucoside by comparing the spectral data with the published spectral data (Hallett & Parks, 1951; Gills, 1964).

Fraction E (2.6 g, $CHCl_3$:MeOH, 60:40) was subjected to C_{18} MPLC using mixtures of methanol and water. A total of 20 fractions in 25 ml portions were collected. Fractions eluted with MeOH:H_2O, 30:70, and 50:50 with similar TLC profiles were mixed and recrystallized from aqueous methanol to give D-mannitol (20 mg).

2.4. Lipid peroxidation inhibitory assay

The compounds **1**–**5** were tested *in vitro* for their potential to inhibit the oxidation (Jayaprakasam, Vanisree, Zhang, Dewitt, & Nair, 2006) of large unilamellar vesicles (LUVs). Phospholipid 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (SLPC) in $CHCl_3$ (10 mg/ml) and fluorescence probe 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl] phenylpropionic acid (DPH-PA) in DMF (mg/ml) were mixed and evaporated under reduced pressure to yield a residue. This was freeze–thawed in MOPS buffer and homogenized with an extruder (Avestin Inc., Ottawa, ON, Canada) to yield LUVs. The final assay volume was 2 ml, consisting of 100 μL HEPES buffer (50 mM HEPES and 50 mM TRIS), 200 μl NaCl (1 M), 1.64 ml of N_2-purged water, 20 μl of test sample or DMSO and 20 μl of liposome suspension. The peroxidation was initiated by the addition of 20 μl $FeCl_2 \cdot 4H_2O$ (0.5 mM). The fluorescence was monitored at 0, 1, 3 and every 3 min up to 21 min using a Turner Model 450 Digital Fluorometer (Branstead Thermolyne, Debuque, IA). The decreases in relative fluorescence intensity over the time, indicated the rate of peroxidation. The percentage of inhibition was calculated with respect to DMSO control. All extracts and compounds were tested at 250 μg/ml and 5 μg/ml, respectively. Positive controls BHA, BHT, and TBHQ were tested at 1 μM.

2.5. Cyclooxygenase inhibitory assay

Cyclooxygenase enzyme inhibitory assay of the extracts and compounds were carried out using COX-1 and COX-2 enzymes according to the previously published procedures (Jayaprakasam et al., 2006). The rate of oxygen consumption during the initial phase of the enzyme-mediated reaction, with arachidonic acid as substrate was measured using a Model 5300 biological oxygen monitor (Yellow Spring Instrument, Inc., Yellow Spring, OH). The test compounds, extracts and positive controls were dissolved in DMSO and an aliquot of 10 μl of each was added to the reaction chamber containing 0.6 mL of Tris buffer (0.1 M, pH 7) and, 1 mM of phenol, hemoglobin (17 μg). COX-1 or -2 enzymes (10 μl) was added to the chamber and incubated for 3 min. The reaction was initiated by the addition of arachidonic acid (10 μl of 1 mg/ml solution). Instantaneous inhibition was measured by using Quick Log Data acquisition and control computer software (Strawberry Tree Inc., Sunnyvale, CA, USA). The percent inhibition was calculated with respect to DMSO control. Each sample was assayed twice, the experiment repeated twice and the standard deviation was calculated. Aspirin, Celebrex and Vioxx were used as positive controls.

3. Results and discussion

The underground and aerial parts of *A. leptopus* are used in food and preparations of herbal medicines in several parts of the world. The main objective of the present study was to identify the bioactive components in *A. leptopus* extracts and resulted in the isolation and characterization of a number of compounds that are predominant in the dried aerial parts of this plant (Fig. 1). To achieve this, we extracted the dried aerial parts of *A. leptopus* sequentially with hexane, ethyl acetate and methanol. The resulting extracts, devoid of organic solvents, were evaluated for their LPO and COX enzyme inhibitory activities at 250 μg/ml concentration according to established procedures in our laboratory (Jayaprakasam et al., 2006). This bioassay-directed approach revealed that the methanolic extract was most active and inhibited LPO by 89% whereas, hexane and ethyl acetate extracts showed only 10% and 4% inhibitions, respectively (Fig. 2). Also, the methanolic extract gave 50.4% and 72.5% inhibition of COX-1 and -2 enzymes, respectively, at 250 μg/ml (Fig. 3).

The bioactive MeOH extract was fractionated by using MPLC silica gel column and afforded fractions A–E. Fractions B, C, and D were found to inhibit LPO and COX enzymes and hence were purified further by MPLC silica gel column chromatography, followed by silica gel preparative TLC.

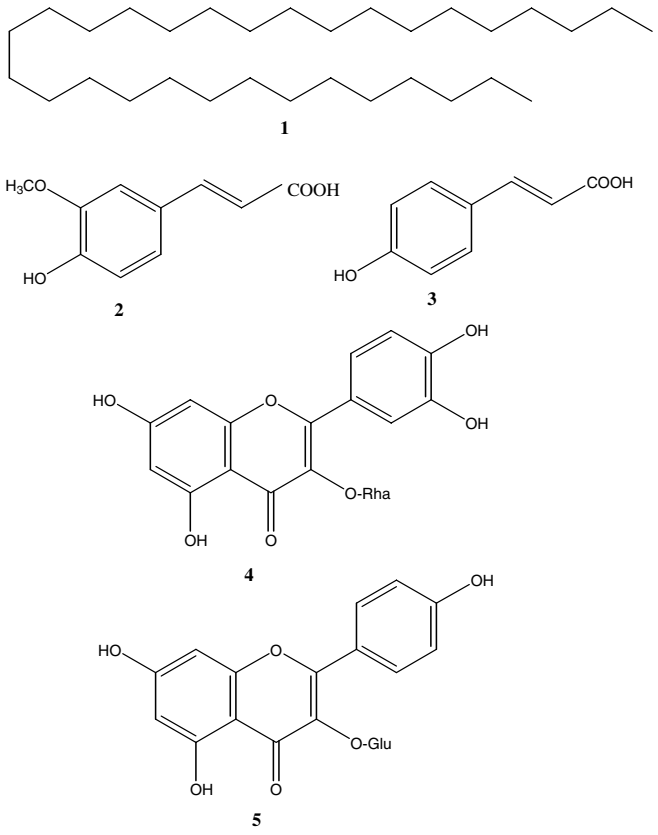

Fig. 1. Structures of compounds isolated from *Antigonon leptopus*.

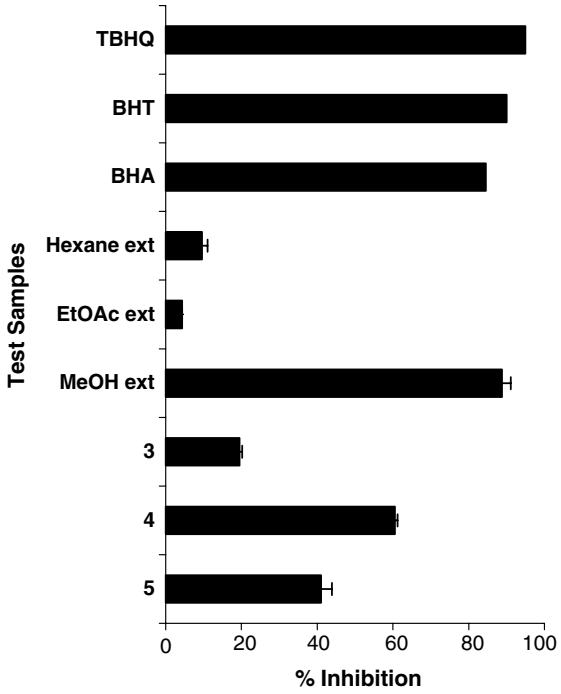

Fig. 2. Inhibition of lipid peroxidation by *Antigononleptopus*. Extracts were tested at 250 μg/ml concentration. Pure compounds were tested at a concentration of 5 μg/ml. Commercial antioxidants BHA, BHT and TBHQ were used as positive controls and tested at 10 μM concentration. Lipid peroxidation was initiated by Fe^{2+} and the rate of decrease in fluorescence intensity was monitored over 21 min. The percent inhibition was calculated with respect to solvent control (DMSO) and values represent mean ±SD ($n = 2$).

The chromatographic purification of fraction B resulted in the isolation of 3-methoxy-4-hydroxycinnamic acid (ferulic acid, compound **2**), as confirmed by the comparison of its spectral data with the reported values in the literature (Wu et al., 1998). Ferulic acid is one of the most abundant phenolic acids in vegetables, fruits and seeds of a number of edible plants. Some of the significant biological activities of ferulic acid and its analogues have been reported recently from our laboratory (Jayaprakasam et al., 2006). Further purification of fraction C gave compound **3** along with the ubiquitous β-sitosterol glucoside. Compound **3** was identified as 4-hydroxycinnamic acid based on the comparison of its spectral data with that of the published spectral data (Owen et al., 2000). Like other phenolic acids, hydroxycinnamic acid is also widely distributed in fruits, vegetables, and is a constituent in beverages (Robbins, 2003). Compound **3**, at 5 μg/ml, showed about 20% inhibition of LPO (Fig. 2) and 30% inhibition at 25 μg/ml concentration. Both ferulic and 4-hydroxycinnamic acids have attracted considerable attention due to their reported health benefits (Komali, Zheng, & Shetty, 1999; Laranjinha, Vieira, Madeira, & Almeida, 1995; Nardini et al., 1995; Roche, Dufour, Mora, & Dangles, 2005).

The repeated purification of fraction D afforded compounds **4** and **5** (Fig. 1) and their structures were identified as quercetin-3-rhamnoside and kaempferol-3-glucoside,

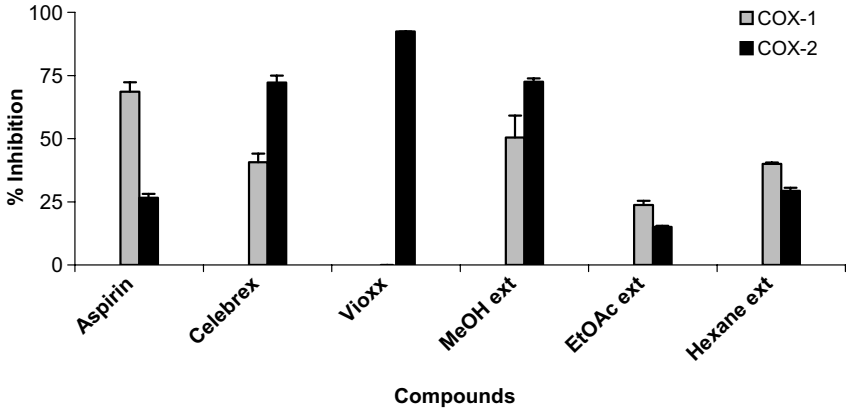

Fig. 3. Inhibition of COX-1 and -2 enzymes by *Antigonon leptopus*. Extracts were tested at 250 μg/ml concentration. Pure compounds were tested at a concentration of 25 μg/ml. Commercial standards Aspirin, Celebrex and Vioxx were used as positive control and tested at 60 μM, 26 nM, and 32 nM concentrations, respectively. DMSO was used as solvent control. Percent inhibition was calculated with respect to DMSO control. Vertical bars represent average ($n = 2$) of two experiments ±SD.

respectively (Gills, 1964; Hallett & Parks, 1951). Both quercetin and kaempferol are common antioxidant flavonoids found in edible plants and are usually present in glycosylated forms. Natural antioxidants are important ingredients that facilitate the prevention of the oxidative deterioration of foods. Foods containing these antioxidants are expected to have preventive activity against oxidation-related diseases. Therefore, we have evaluated quercetin-3-rhamnoside (compound **4**) and kaempferol-3-glucoside (compound **5**) isolated from *A. leptopus* for LPO inhibitory activity. At 5 μg/ml concentration, compound **5** exerted about 61% of inhibition against LPO (Fig. 2). However, compound **4** showed only 41% of LPO inhibitory activity when tested at 5 μg/ml. The positive controls BHA, BHT and TBHQ were tested at 10 μg/ml concentrations.

Inflammation plays a role in many diseases, such as arthritis, heart disease, stroke, and even in Alzheimer's disease. The COX enzymes catalyze the conversion of arachidonic acid to prostaglandins, which are responsible for the onset of inflammation in the body. It has been proven that selective COX-2 inhibitors can serve as better anti-inflammatory products. Our interest in COX enzyme inhibitors from functional foods prompted us to investigate the inhibitory activities of *A. leptopus* compounds against COX-1 and COX-2 enzymes. In the preliminary evaluations, compounds **3**–**5** showed excellent inhibitions of COX enzymes at 25 μg/ml concentrations. The results showed that compound **3** inhibited COX-1 and -2 enzymes by 64.7% and 87.4%, respectively. The inhibitory concentration at 50% (IC_{50}) of 4-hydroxycinnamic acid for COX-1 and -2 enzymes was 17.4% and 8.57 μg/ml, respectively (Fig. 4a and 4b). Similarly, compound **5** inhibited COX-1 and -2 enzymes by 38.5% and 90.2%, respectively, whereas, compound **4** showed inhibitions by 16.9% and 88.8%, respectively, at 25 μg/ml concentrations. The IC_{50} values of compounds **4** and **5** for COX-1 and -2 were 68.9, 36.3 and 7.86, 6.78 μg/ml, respectively (Fig. 4a and b). The positive controls, Aspirin, Celebrex and Vioxx were tested at

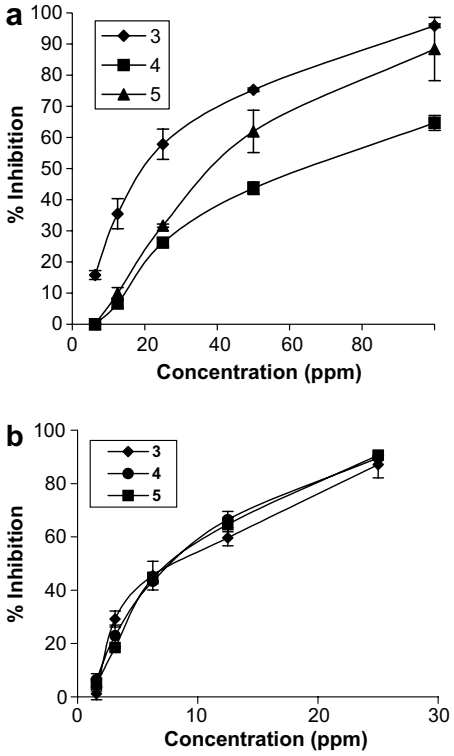

Fig. 4. (a) Dose response curves for the inhibition of COX-1 enzyme by compounds **3**, **4**, and **5**. DMSO was used as solvent control. (b) Dose response curves for the inhibition of COX-2 enzyme by compounds **3**, **4**, and **5**. DMSO was used as solvent control. Percent inhibition was calculated with respect to DMSO control and the vertical bars represent average ($n = 2$) of two experiments ±SD.

60 μM (108 μg/ml), 26 nM (1 μg/ml), and 32 nM (1 μg/ml) concentrations, respectively. The different concentrations of positive controls used in the assay were to obtain an inhibitory concentration (IC) between 50% and 100% since these drugs inhibit the COX enzymes at different levels. Compounds **2**–**5** were isolated for the first time from *A.*

leptopus. Compound **1**, although present in appreciable quantities in the plant extract, did not exhibit LPO and COX inhibitory activities when tested at 25 μg/ml. Due to solubility problems, we were unable to test this compound at higher concentrations. The methanolic extract of this plant material contained quercetin-3-rhamnoside (**4**) as the major active compound.

In conclusion, the present study reveals that the total bioactivity of the methanolic extract may result from the phenolics present in it. Based on previous reports and the present study, the compounds isolated from the aerial parts of *A. leptopus* plant have the ability to scavenge reactive oxygen species and reduce oxidative stress *in vivo*. In addition to this scavenging effect, these compounds may also enhance the endogenous defense systems. It is implicated that polyphenols exert their activities by many pathways such as free radical scavenging, oxygen radical absorbance and chelating of the metal ions. In addition, they also exhibited significant COX inhibitory activities, especially the COX-2 enzyme. Inflammation is a concern to all and anti-inflammatory agents as a functional food, are of great interest. Therefore, products prepared from traditional plants such as *A. leptopus* and its use as ingredients in herbal supplements are probably appealing to consumers.

References

Bome, R. F. (1995). *Non-steroidal anti-inflammatory drugs* (2nd ed.). *Principles of medicinal chemistry*. USA: Williams and Wilkins.

Charmaine, S. (1998). *Asia food. Encyclopedia of Asian food* (Periplus editions). Australia: New Holland Publishers Pty Ltd.

Chistokhodova, N., Nguyen, C., Calvino, T., Kachirskaia, I., Cunningham, G., & Miles, D. H. (2002). Antithrombin activity of medicinal plants from central Florida. *Journal of Ethnopharmacology, 81*, 277–280.

Gills, S. (1964). Studies on the chemical composition of trifolium arvense L. IV. Isolation and identification of kaempherol-3-glycoside. *Acta Poloniae Pharmaceutica, 21*, 287–290.

Hallett, F. P., & Parks, L. M. (1951). A note on the isolation of quercitrin from *Euphorbia pilulifera*. *Journal of the American Pharmaceutical Association, 40*, 56–57.

Halliwell, B., Aeschbach, R., Loliger, J., & Aruoma, O. I. (1995). The characterization of anti-oxidants. *Food Chemical Toxicity, 33*, 601–617.

Halliwell, B., & Gutteridge, J. M. C. (1995). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal, 219*, 1–4.

Hasan, M., & Burdi, D. K. (1991). Diterpene fatty acid esters from *Leucas nutans*. *Journal of Natural Products, 54*(5), 1444–1446.

Henry, M., & Harris, K. S. (2002). *LMH Official dictionary of Jamaican herbs & medicinal plants and their uses*. LMH Publishing Ltd.

Jayaprakasam, B., Vanisree, M., Zhang, Y., Dewitt, D. L., & Nair, M. G. (2006). Impact of alkyl esters of caffeic and ferulic acids on tumor cell proliferation, cyclooxygenase enzyme and lipid peroxidation. *Journal of Agricultural and Food Chemistry, 54*(15), 5375–5381.

Komali, A. S., Zheng, Z., & Shetty, K. (1999). A mathematical model for the growth kinetics and synthesis of phenolics in oregano (*Origanum vulgare*) shoot cultures inoculated with *Pseudomonas* species. *Process Biochemistry, 35*, 227–235.

Lans, C. A. (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *Journal of Ethnobiology and Ethnomedicine, 2*, 1–11.

Laranjinha, J., Vieira, O., Madeira, V., & Almeida, L. (1995). Two related phenolic antioxidants with opposite effects on vitamin E content in low density lipoproteins oxidized by ferrylmyoglobin: Consumption vs regeneration. *Archives of Biochemistry and Biophysics, 323*, 373–381.

Marnett, L. J. (2002). Oxy radicals, lipid peroxidation and DNA damage. *Toxicology*, 219–222.

Mitchell, S. A., & Ahmad, M. H. (2006). A review of medicinal plant research at the University of the West Indies, Jamaica. *West Indian Medicinal Journal, 55*(4), 243–253.

Nardini, M., D'Aquino, M., Tomassi, G., Gentili, V., Di Felice, M., & Scaccini, C. (1995). Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biology and Medicine, 19*, 541–552.

Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Spiegelhalder, B., & Bartsch, H. (2000). The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer, 36*(10), 1235–1247.

Raju, A. J. S., Raju, V. K., Victor, P., & Naidu, S. A. (2001). Floral ecology, breeding system and pollination in *Antigonon leptopus* L. (Polygonaceae). *Plant Species Biology, 16*, 159–164.

Robbins, R. J. (2003). Phenolic acids in foods: An overview of analytical methodology. *Journal of Agricultural Food Chemistry, 51*, 2866–2887.

Roche, M., Dufour, C., Mora, N., & Dangles, O. (2005). Antioxidant activity of olive phenols: Mechanistic investigation and characterization of oxidation products by mass spectrometry. *Organic and Biomolecular Chemistry, 3*, 423–430.

Smith, W. L., De Witt, D. L., & Garavito, R. M. (2000). Cyclooxygenases: Structural, cellular, and molecular biology. *Annual Review of Biochemistry, 69*, 145–182.

Williams, D. E., Dashwood, R. H., Hendricks, J. D., & Bailey, G. S. (1989). In S. L. Taylor & R. A. Scanlan (Eds.), *Food toxicology – A perspective on relative risks* (pp. 101–150). New York: Marcel Dekker.

Wu, Tian-Shung., Chan, Yu-Yi., & Leu, Yann-Lii. (2000). The constituents of the root and stem of *Aristolochia heterophylla* hemsl. *Chemical and Pharmaceutical Bulletin, 48*(3), 357–361.

Wu, Tian-Shung., Leu, Yann-Lii., & Chan, Yu-Yi. (1998). Constituents of the leaves of *Aristolochia kaempferi*. *Chemical and Pharmaceutical Bulletin, 46*(10), 1624–1626.