Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 485–490
Food Chemistry
www.elsevier.com/locate/foodchem

Terpenoids from Stinking toe (*Hymneae courbaril*) fruits with cyclooxygenase and lipid peroxidation inhibitory activities

Bolleddula Jayaprakasam [a], Ruby L. Alexander-Lindo [b], David L. DeWitt [c], Muraleedharan G. Nair [a,*]

[a] *Bioactive Natural Products and Phytoceuticals, Department of Horticulture and National Food Safety and Toxicology Center, East Lansing, MI 48824, USA*
[b] *Department of Basic Medical Sciences, University of the West Indies, Mona, Kingston, Jamaica*
[c] *Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA*

Received 28 September 2006; received in revised form 29 January 2007; accepted 2 April 2007

Abstract

Stinking toe (*Hymenaea courbaril*), also called Jatoba and Kerosene tree, is a medicinal plant commonly found in the central and South American countries. In the Caribbean, Mexico and Brazil, the powdery sweet dust of its fruit is consumed for energy. The chemical examination of the yellowish sweet powder of the fruit yielded sucrose and linolenic acid as major compounds. The pods yielded the labdane diterpenoids crotomachlin (**1**), labd-13*E*-en-8-ol-15-oic acid (**2**), labdanolic acid (**4**), (13*E*)-labda 7, 13 dien-15-oic acid (**5**) and labd-8 (17), 13*E*- dien-15-oic acid (**6**), along with the sesquiterpene, spathulenol (**7**), as confirmed by ^{1}H and ^{13}C NMR spectral studies. The methyl ester of labd-13*E*-en-8-ol-15-oic acid (**3**) was also characterized during the purification of compound **5**. The total amount of these terpenoids in the fruit was about 0.1% (w/w) of the dried fruit. Compounds **1**–**5** and **7** were assayed for anti-inflammatory activity using cyclooxygenase-1 (COX-1) and -2 (COX-2) enzymes. At 100 ppm, compounds **3** and **4** showed selective COX-2 enzyme inhibition. Also, compounds **1**, **2** and **5** inhibited lipid peroxidation by 46%, 48% and 75%, respectively, at 100 ppm. These compounds were isolated from this fruit and their COX and lipid peroxidation inhibitory activities are reported for the first time in this paper.

Keywords: *Hymenaea courbaril*; Sucrose; Diterpenoids; Cyclooxygenase inhibition; Lipid peroxidation inhibition

1. Introduction

The genus *Hymenaea* belongs to the family Caesalpiniaceae and is widely distributed in the Caribbean and South America. Only *Hymenaea courbaril* and *Hymenaea martaina* yield fruits among the fifteen *Hymenaea* spp. (Lee & Langenheim, 1975) known. The fruits are 10–20 cm in length and 4–6 cm in diameter and contain a powdery pulp or flour. They are eaten by the local population and used as an ingredient in porridge, regional dishes and beverages (Arckcoll, 1984; Witsberger, Current, & Archer, 1982). A recent study showed that the fruit flour is rich in fibre and can be used in the preparation of healthy snacks (Chang, Silva, Gutkoski, Sebio, & Da Silva, 1998). Very little research has been carried out on the bioactivity and chemistry of the edible portion of the fruit.

The bark, resin and leaves of the *H. courbaril* plant have been used for the treatment of arthritis, asthma, bronchitis and rheumatism (Duke & Wain, Medicinal Plants of the World. http://www.hort.purdue.edu/newcrop/duke_energy/Hymenaea_courbaril.html#Folk%20Medicine). The exudates of the gum resin from *H. courbaril* were used as incense (Little & Wadsworth, 1964) and showed potent lipoxygenase inhibitory activity (Braga, Wagner, Lombardi, & DeOliveira, 2000). These resins are rich sources of diterpene carboxylic acids. Earlier investigations have

* Corresponding author. Tel.: +1 517 432 3100x141; fax: +1 517 432 2310.
E-mail address: nairm@msu.edu (M.G. Nair).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.04.004

revealed several diterpenoids from the leaves and pod extracts of *H. courbaril* (Abdel-Kader et al., 2002; Khoo & Oehlschlager, 1973; Marsaioli, Leitao Filho, & De Paiva Campello, 1975; Nogueira, Shephered, Laverde, Marsaioli, & Imamura, 2001). However, the biological activities of the constituents of *H. courbaril* have not been reported. The preliminary bioassays conducted in our laboratory, on the methanolic extract of *H. courbaril* fruits, showed strong COX enzyme and lipid peroxidation inhibitory activities.

Cyclooxygenase enzymes play a critical role in inflammatory disorders. The two isozymes of cyclooxygenases (COX-1 and -2) catalyze a rate-limiting step in prostaglandin synthesis. These enzymes have been a target for drug design to treat inflammations and related disorders. COX-1 is constitutively expressed in cells and it maintains the integrity of gastrointestinal mucosa whereas COX-2 is induced by external stimuli. Inhibition of COX-1 enzyme could result in gastrointestinal damage and ulcers. Selective COX-2 inhibitors are therefore preferred over COX-1 inhibitors to alleviate inflammation (Smith, DeWitt, & Garavito, 2000). However, several selective COX-2 inhibitors have been found to cause cardiovascular problems (Mukherjee, Nissen, & Topol, 2001). The intermediates, e.g. superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$), formed during the conversion of respiratory oxygen to H_2O, are highly reactive and toxic. These intermediates induce lipid peroxidation, cross-linking of proteins, DNA scissions and decreased mitochondrial function. Some phytochemicals have been reported to alleviate the damage caused by these reactive species (Ames, Shigenaga, & Hagen, 1993). Hence, much attention has been focussed on COX-2 enzyme and lipid peroxidation inhibitors present in a variety of edible fruits and vegetables. Our bioassay-guided research on *H. courbaril* fruits yielded labdane diterpenoids and a sesquiterpene with COX enzyme and lipid peroxidation inhibitory activities. Among the terpenoids reported in this paper, compounds **1** and **7** are isolated for the first time from this plant.

2. Materials and methods

2.1. General experimental procedure

^1H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on an INOVA Varian VRX 500 instrument. The chemical shifts (δ) were measured in DMSO-d_6 or $CDCl_3$. The silica gel used for MPLC (medium pressure liquid chromatography) was Merck Silica gel 60 (35–70 μm particle size). The PTLC (preparative thin layer chromatography) plates (20 × 20, 500 μm) were purchased from Analtech, Inc. (Newark, DE). ACS grade solvents were used in the purification of compounds. The COX-1 enzyme was prepared from ram seminal vesicles purchased from Oxford Biomedical Research, Inc (Oxford, MI) as described previously (Sivarajah, Lasker, & Eling, 1981). The COX-2 enzyme was prepared from insect cells cloned with human PGHS-2 (prostaglandin endoperoxide H synthase-2) enzyme. Preparative HPLC (high pressure liquid chromatography) (Japan Analytical Industry Co. model LC-20) was used with a JAIGEL-ODS-C_{18} column for the separation of compounds. Positive controls for lipid peroxidation assay used were butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhdroquinone (TBHQ), purchased from Sigma–Aldrich Co. (St Louis, MO) and tested at 1 ppm. The lipid, 1-stearoyl 2-linoleoyl *sn*-glycerol 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). Acetyl salicylic acid, ibuprofen and rofecoxib were used as positive controls in COX enzyme inhibitory assays.

2.2. Plant material

The fruits of *H. courbaril* were collected at Jacks Hill, Kingston, Jamaica in June 2003. A voucher specimen is available at the herbarium in the Department of Basic Medical Sciences, University of the West Indies, Jamaica.

2.3. Extraction and isolation

The fruits were broken, and the pods and the edible sweet powder from the seeds were separated. The edible powder (300 g) was extracted sequentially with *n*-hexane (3 × 500 ml), EtOAc (3 × 500 ml) and MeOH (3 × 500 ml). The *n*-hexane (300 mg) and EtOAc (200 mg) extracts were identical in nature by TLC and hence were combined. The purification of the combined extracts by column chromatography (silica gel) using *n*-hexane and EtOAc (8:2) as the mobile phase yielded a major compound, linolenic acid (200 mg), as identified by ^1H, ^{13}C NMR and GCMS. The MeOH extract (75 g) contained a single major compound, as indicated by TLC. Therefore, it was crystallized from MeOH to yield colourless crystals and identified as sucrose by ^1H and ^{13}C NMR spectral analyses.

The pulverized pods of *H. courbaril* (500 g) were extracted sequentially with $CHCl_3$ (3 × 1 l) and MeOH (3 × 1 l). The evaporation of solvent under reduced pressure yielded 8.5 and 6.4 g extracts, respectively. The $CHCl_3$ (4.5 g) extract was fractionated by silica gel medium pressure liquid chromatography (MPLC, Sanki Engineering Ltd., model LBP-V pump operating at 10–15 psi) (ACE glass, 40 mm i.d.× 30 mm length) using *n*-hexane, $CHCl_3$, MeOH as mobile phases under gradient conditions; 120 fractions were collected and combined based on TLC, to yield fractions A (500 mg), B (600 mg), C (1 g), D (1.2 g) and E (900 mg). Purification of fraction B by CC, followed by PTLC ($CHCl_3$:MeOH, 97:3, v/v), gave compound **7** (400 mg). Similarly, purification of fraction C by CC and PTLC ($CHCl_3$:MeOH, 90:10, v/v) afforded compound **1** (30 mg). Fractionation of D (420 mg) by reverse phase prep-HPLC (Japan Analytical Industry Co. model LC-20)

with JAIGEL-ODS-C_{18} (MeOH:H_2O, 88:12, v/v) gave fr. 1 (100 mg), fr. 2 (200 mg) and fr.3 (50 mg). The fr. 2 was repeatedly purified by prep. HPLC, using MeOH:H_2O (90:10, v/v) as the mobile phase, and gave pure compound **2** ($R_f = 42$ min, 45 mg). Methylation of fr. 3 using diazomethane and subsequent purification gave compound **3**. Purification of fr. E (300 mg) by CC and PTLC gave compounds **4** (10 mg), **5** (15 mg) and **6** (0.8 mg), respectively.

2.4. Compounds 1–7

2.4.1. General

Compounds **1**–**7** were identified as crotomachlin (**1**) (Herlem, Huu, & Kende, 1994; Teramoto et al., 1996), labd-13*E*-en-8-ol-15-oic acid (**2**), (Imamura, Marsaioli, Barata, & Ruveda, 1977), labd-13*E*-en-8-ol-15-oic acid methyl ester (**3**) (Castro, Salido, Altarejos, Nogueras, & Sanchez, 2002), labdanolic acid (**4**) (Castro et al., 2002), (13*E*)-labd 7, 13 dien-15-oic acid (**5**) (Imamura et al., 1977), and labd-8 (17), 13*E*-dien-15-oic acid (**6**) (Carman & Duffield, 1995) and spathulenol (**7**) (Krebs, Rakotoarimanga, & Habermehl, 1990), respectively (Fig. 1), by ^1H and ^{13}C NMR spectral studies and by comparison with the published spectral results. Compounds **1** and **7** were isolated for the first time from this plant and hence their spectral data are given below.

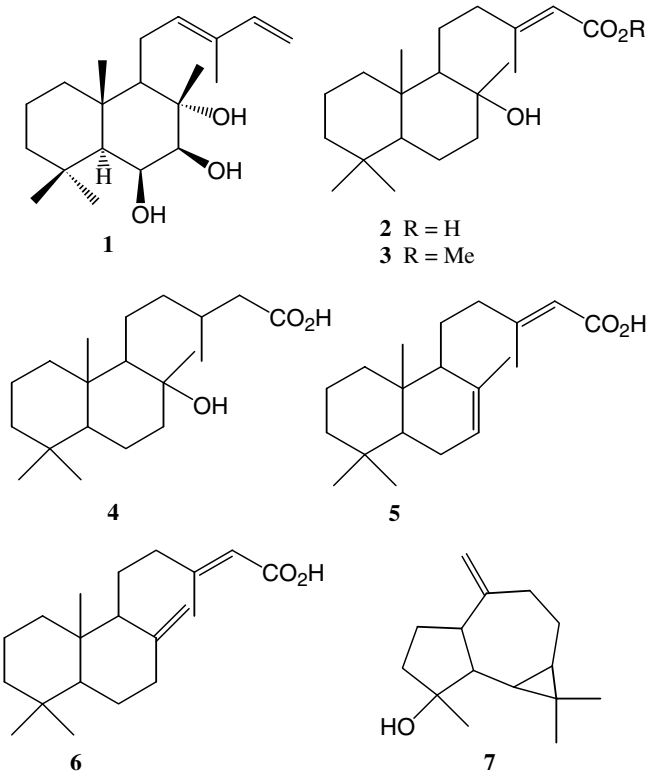

Fig. 1. Chemical structures of compounds **1**–**7**.

2.4.2. Compound (1)

^1H NMR (300 MHz, DMSO-d_6): δ 6.28 (1H, dd, $J = 10.5$, 17.1 Hz, H-14), 5.55 (1H, t, $J = 6.9$ Hz, H-12), 4.98 (1H, d, $J = 17.1$ Hz, H-15a), 4.83 (1H, d, $J = 10.5$ Hz, H-15b), 4.43 (1H, br d, $J = 3.9$ Hz, H-7), 4.10 (1H, br s, H-6), 2.37 (1H, m, H-11a), 2.13 (1H, t, $J = 5.5$ Hz, H-11b), 1.67 (3H, s, Me-16), 1.13 (3H, s, Me-17), 1.12 (6H, s, Me-19 and 20), 0.90 (3H, s, Me-18). ^{13}C NMR (300 MHz, DMSO-d_6): δ 141.9 (C-14), 138.3 (C-13), 130.5 (C-12), 109.8 (C-15), 80.0 (C-7), 75.1 (C-8), 70.5 (C-6), 60.3 (C-9), 55.07 (C-5), 43.3 (C-3), 41.8 (C-10), 38.6 (C-1), 33.7 (C-4), 33.1 (C-18), 23.6 (C-16), 23.0 (C-11), 19.1 (C-17), 18.2 (C-2), 16.4 (C-19), 11.7 (C-20).

2.4.3. Spathulenol (7)

^1H NMR (500 MHz, DMSO-d_6): δ 4.61 (2H, br s, H-15), 4.0 (1H, s, OH-11), 2.35 (1H, dd, $J = 6.5$ Hz, H–6a), 2.0 (1H, m, H-8), 1.98–1.0 (7H, m), 1.14 (3H, s, Me-12), 1.0 (3H, s, Me-14), 0.96 (3H, s, Me-13), 0.92 (1H, m, H-5a), 0.64 (1H, m, H-4), 0.43 (1H, t, $J = 8.0$ Hz). ^{13}C NMR (500 MHz, DMSO-d_6): δ 153.1 (C-7), 105.9 (C-15), 78.6 (C-11), 53.0 (C-8 and 1), 41.3 (C-10), 38.4 (C-6), 29.7 (C-4 and 2), 28.5 (C-9), 26.7 (C-7), 26.3 (13), 25.9 (C-5), 24.3 (C-12), 19.6 (C-3), 16.2 (C-14).

2.5. Cyclooxygenase enzyme inhibitory assay

The anti-inflammatory assay was conducted using COX-1 and -2 enzymes according to the published procedure (Jayaprakasam, Zhang, & Nair, 2004). In brief, COX enzymes were diluted (1:1) with Tris buffer (pH 7 ± 0.5, 10–30 μl) and the test compounds dissolved in DMSO, at 100 ppm final concentration, were added to the chamber containing the assay mixture (3 ml of 0.1 M Tris HCl, pH 7 ± 0.5, 1 mmol phenol and 17 μg of hemoglobin). The mixture was incubated for 2 min and the reaction was initiated by the addition of arachidonic acid (10 μl of 1.64 μM solution). The rate of O_2 uptake was measured using an oxygen electrode (Instech laboratories, Plymouth Meeting, PA) attached to a biological oxygen monitor (Yellow Spring Instrument, Inc., Yellow Spring, OH) at 37 °C. The oxygen uptake was measured by using Quick Log Data acquisition and control computer software (Strawberry tree Inc., Sunnyvale, CA, USA). The percent inhibition of the enzyme by test compounds was calculated with respect to DMSO solvent control and standard deviation was calculated for $n = 2$. Positive controls, acetyl salicylic acid, ibuprofen and rofecoxib, were tested at 180, 2.1, 1.67 ppm, respectively.

2.6. Lipid peroxidation inhibitory assay

Compounds **1**–**5** and **7** were assayed for lipid peroxidation inhibitory activity by a published procedure (Jayaprakasam, Strasburg, & Nair, 2004). The liposome suspension was prepared by mixing the phospholipid 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (SLPC) and

a fluorescence probe [3-[*p*-(6-phenyl)-1,3,5-hexatrienyl] phenylpropionic acid (DPH-PA) (Arora, Nair, & Strasburg, 1998). The peroxidation was initiated by the addition of 20 μl of $FeCl_2 \cdot 4H_2O$ (0.5 mM) to the assay mixture [HEPES (100 μl), 1 M NaCl (200 μl), N_2-sparged water (1.64 ml), test sample or DMSO (20 μl)] and 20 μl of liposome suspension. The fluorescence was monitored at 0, 1, 3 and every 3 min up to 21 min using a Turner Model 450 Digital fluorometer. The decrease in fluorescence intensity over time (21 min) indicated the rate of peroxidation ($n = 2$). DMSO was used as solvent control and BHA, BHT and TBHQ as positive controls.

3. Results and discussion

The *H. courbaril* fruits are dark in colour with hard pods. They contain dark, hard and woody seeds, coated with an ample supply of yellowish sweet powder. In this study, we have analyzed the edible powder and seed pod separately. The combined *n*-hexane and EtOAc extracts yielded, linolenic acid (200 mg) as a major compound. The MeOH extract of the edible powder contained a single sugar and crystallization of it from MeOH yielded pure sucrose (250 mg/g of dry powder). Its identity was established by ^{1}H and ^{13}C NMR spectral data in addition to silica gel TLC comparison with an authentic sample of sucrose. The non-extractable portion of the edible powder by MeOH was not soluble in DMSO, water or acidic water and hence was not studied further. This indicated that the composition of the edible powder was, about, 25% of sucrose, 0.067% linolenic acid and the rest as fibrous plant material. There was TLC evidence of trace quantities of other compounds. Therefore, we have also investigated the pods of *H. courbaril*, although they were not edible, and isolated terpenoids as bioactive compounds. These compounds were in the trace quantities detected by TLC in the edible portion of the fruit.

Compound **1** was obtained as an amorphous powder. The ^{1}H NMR spectrum of **1** showed a doublet (δ 6.28), two doublets (δ 4.98 and 4.83) and a triplet at δ 5.55, which indicated two olefinic bonds in the molecule. It showed signals for the protons attached to oxygenated carbons at δ 4.10 and 4.43 in addition to five methyl groups. The ^{13}C NMR spectrum of compound **1** displayed twenty carbon signals and indicated a diterpenoid structure for the molecule. The DEPT spectrum showed five methyls, five methylenes, six methines, and four quartenary carbons in **1**. The carbon signals at δ 141.9, 138.3, 130.5 and 109.8 supported the two double bonds and the signals at δ 80.0, 75.1 and 70.5 revealed the presence of three hydroxyl groups in the molecule. In addition to the methyl signals at δ 33.1, 23.6, 19.1, 16.4 and 11.7, the methylene carbons appeared at δ 38.6, 18.2, 43.3 and 23.0 and the methine carbons at δ 60.3 and 55.07, respectively. The above data suggested the structure of compound **1** to be that of crotomachlin (Herlem et al., 1994; Teramoto et al., 1996). Since the H–5 in the molecule appeared at δ 1.54, its geometry was established as "α". The correlations of H-6 to H-7 and H-7 to H-16 in its NOESY spectrum revealed that the orientations of hydroxyl groups at 6, 7 and 8 positions were β, β and α, respectively.

Compound **7**, colourless oil, gave ^{1}H NMR signals for an exomethylene moiety at δ 4.61. A D_2O exchangeable proton at δ 4.0 indicated the presence of a hydroxyl group in the molecule. In addition, its spectrum also exhibited three methyl signals at δ 1.14, 1.0 and 0.96. The ^{13}C NMR spectrum of **7** displayed signals for 15 carbons and indicated a sesquiterpenoid structure. The two olefinic carbon signals at δ 153.1 and 105.9 were assigned to the exocyclic double bond. Apart from the carbons in the double bond, there were signals for three methyls (δ 26.3, 24.3 and 16.2), four methylenes (δ 25.9, 38.4, 28.5 and 41.3), four methine (δ 29.7 and 53.0) and two quaternary carbons (δ 19.6 and 78.6) in compound **7**. The spectral data of compound **7** were in agreement with the published spectral data of spathulenol (Krebs et al., 1990).

Plant products have played an important role in maintaining human health for thousands of years, and have been used as ingredients in seasonings, beverages, cosmetics and medicines. Among the phytochemicals, terpenoids are one of the major classes of plant natural products found widely in dietary plants, and are considered nontoxic (Akihisa, Yasukawa, & Tokuda, 2003). Some of these terpenoids, known to be beneficial to human health, are limonene, carvone or carveol (monoterpenes), retinoids (diterpenes) and carotenoids, lutein, lycopene, zeaxanthine and cryptoxanthine (tetraterpenes) (Karl-Heinz & Ibrahim, 2003). Among these, the labdane diterpenoids have not been fully explored for their biological activities.

The inhibition of COX enzymes by terpenoids from *H. courbaril* fruits, crotomachlin (**1**), labd-13*E*-en-8-ol-15-oic acid (**2**), labd-13*E*-en-8-ol-15-oic acid methyl ester (**3**), labdanolic acid (**4**), (13*E*)-labda 7, 13 dien-15-oic acid (**5**), and labd-8 (17), 13*E*-dien-15-oic acid (**6**), and spathulenol (**7**) were evaluated at 100 ppm (Fig. 2). Compound **6** was not tested due to the paucity of sample. Crotomachlin (**1**) inhibited COX-1 and -2 enzymes by 29 and 15%, respectively. Interestingly, compounds **3** and **4** inhibited COX-2 enzyme selectively by 40% and 43%, respectively. Similarly, sesquiterpene **7** also showed higher COX-2 enzyme inhibition (54%) when compared to COX-1 enzyme (15%). The labd-13*E*-en-8-ol-15-oic acid (**2**), showed 30% of COX-2 inhibition whereas (13*E*)-labda 7, 13 dien-15-oic acid (**5**) inhibited COX-1 and -2 enzymes by 33% and 53%, respectively. The positive controls, acetyl salicylic acid and ibuprofen, showed 61% and 53%, respectively, of COX-1 inhibition and 24% and 59%, respectively of COX-2 enzyme inhibition at 180 and 2.1 ppm concentration. Rofecoxib is a selective COX-2 enzyme inhibitor, irrespective of the concentration, and was tested at 1.67 ppm to obtain enzyme inhibition >50%. All isolated compounds, except compound **6**, were also tested for their ability to inhibit lipid peroxidation (Fig. 3). Compound **5** was the most active and inhibited lipid peroxidation by 75%,

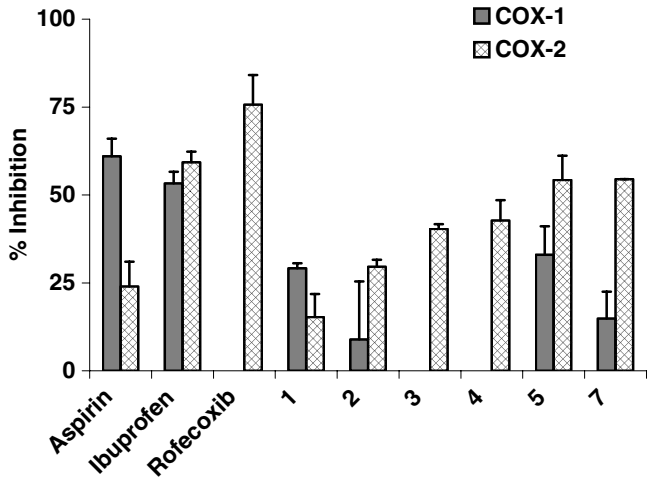

Fig. 2. Cyclooxygenase enzyme inhibitory activities of terpenoids **1**–**5** and **7** at 100 μg/ml. Compounds **3** and **4** did not inhibit COX-1 enzyme. Compound **6** was not tested due to insufficient quantity. Positive controls aspirin, ibuprofen and rofecoxib, were assayed at 180, 2.1 and 1.67 μg/ml, respectively, in order to obtain COX enzyme inhibitions >50%. Acetyl salicylic acid is not a good COX-2 inhibitor, whereas rofecoxib does not inhibit COX-1 at higher concentrations. Vertical bars represent the percent inhibition ±SD ($n = 2$).

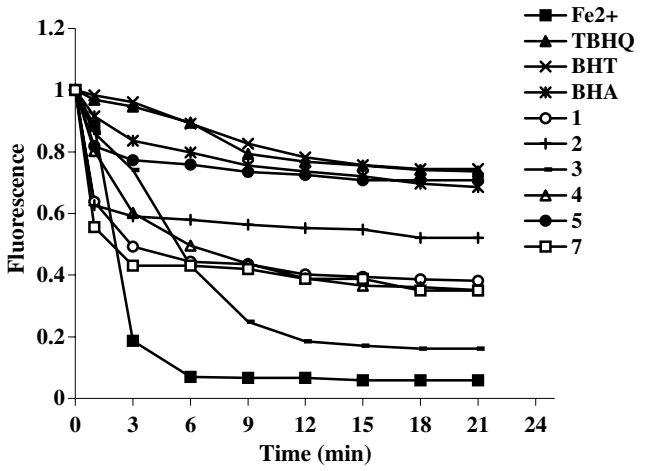

Fig. 3. Lipid peroxidation inhibitory activities of terpenoids **1**–**5** and **7** at 100 μg/ml and commercial antioxidants BHA, BHT and TBHQ at 1 μg/ml. Compound **6** was not tested due to insufficient quantity. The fluorescence intensity was measured over 21 min. The percent inhibition ($n = 2$) was decrease in fluorescence intensity at 21 min with respect to DMSO control.

whereas compounds **1** and **2** showed 46% and 48% of inhibition, respectively. The commercial antioxidants, BHA, BHT and TBHQ, inhibited lipid peroxidation by 85%, 91% and 89%, respectively at 1 ppm concentration. Linolenic acid was isolated as the next major compound, other than sucrose, in *H. courbaril* seed powder. We have reported the COX and lipid peroxidation inhibitory activities of linolenic acid (Henry, Momin, Nair, & Dewitt, 2002) and hence it was not evaluated in the present study.

Compounds **2**–**7** were also evaluated for tumor cell proliferation activity against MCF-7 (breast), SF-268 (central nervous system, CNS) and NCI-H460 (lung), HCT-116 (colon) and AGS (gastric) human tumor cell lines by established procedures in our laboratory (Jayaprakasam, Zhang, et al., 2004). These terpenoids, isolated from *H. courbaril* fruits, did not inhibit the growth of tumor cell lines at 100 ppm (as final concentration in assay wells) and this suggested that they were probably not cytotoxic.

Several studies have demonstrated that COX-2 is over expressed in several pathological conditions, e.g. cancer, diabetes, Alzheimer's disease, and some of the non-steroidal anti-inflammatory drugs (NSAID's) suppress the onset of these diseases (Mitchell & Warner, 1999). The COX-2 inhibitors that are present in medicinal plants and food products are considered by the consumer to be safer than Pharma drugs. For example, curcumin, a plant pigment, inhibits the induction of COX-2 enzyme in colorectal cancers (Surh et al., 2001). Also, resveratrol, a major constituent in grapes, inhibited the TPA (12-*O*-tetradecanoylphorbol-3-acetate) induced COX-2 activity and epigallocatechin gallate (EGCG) inhibited expression of NF-κB activation, a key nuclear transcription factor involved in controlling inflammation and tumorigenesis (Surh et al., 2001).

Terpenoidal compounds are active constituents in several food components. We have recently reported the specific COX-2 inhibitory activity of a series of olean type triterpenoids isolated from the seeds of *Picrorhiza Kurroa*, a medicinal plant used in the traditional Indian system of medicine, Ayurveda (Zhang, DeWitt, Murugesan, & Nair, 2005). We have also reported the inhibition of COX enzymes by a series of fatty acids, including linolenic acid (Henry et al., 2002). The edible portion of *H. courbaril* fruit primarily contained about 25% of sucrose and 0.067% of linolenic acid, in addition to the terpenoids isolated from pods as minor compounds. The bulk of the powder was fibrous material. However, specific COX-2 enzyme and lipid peroxidation inhibitory activities exhibited by the terpenoids and the COX enzyme inhibitory linolenic acid in *H. courbaril* fruits show that consumption of this fruit may be useful in alleviating some of the symptoms associated with inflammation. However, consumption of this fruit may not be advisable to those who are obese and diabetic due to its high sucrose content.

References

Abdel-Kader, M., Berger, J. M., Slebodnick, C., Hoch, J., Malone, S., Wisse, J. H., et al. (2002). Isolation and absolute configuration of *ent*-halimane diterpenoids from *Hymenaea courbaril* from the suriname rain forest. *Journal of Natural Products, 65*, 11–15.

Akihisa, T., Yasukawa, K., & Tokuda, H. (2003). Potentially cancer chemopreventive and anti-inflammatory terpenoids from natural sources. In Atta-ur-Rahman (Ed.). *Studies in natural products chemistry (Part J)* (Vol. 29, pp. 73–126). Amsterdam: Elsevier.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants and degenerative disorders diseases of aging. *Proceedings of National Academy of Sciences, 90*, 7915–7922.

Arckcoll, D. B. (1984). Some leguminous trees providing useful fruits in the north of Brazil. *Pesquisa Agropecuaria Brasilia, 19*, 231–234.

Arora, A., Nair, M. G., & Strasburg, G. M. (1998). Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. *Archives of Biochemistry and Biophysics, 356*, 133–141.

Braga, F. C., Wagner, H., Lombardi, J. A., & DeOliveira, A. B. (2000). Screening of Brazilian plant species for in vitro inhibition of 5-lipooxygenase. *Phytomedicine, 6*, 447–452.

Carman, R. M., & Duffield, A. R. (1995). The optical rotation of some C15-oxygenated labda-8 (17), 13-dienes. *Australian Journal of Chemistry, 48*, 1357–1366.

Castro, J. M., Salido, S., Altarejos, J., Nogueras, M., & Sanchez, A. (2002). Synthesis of ambrox from labdanolic acid. *Tetrahedron, 58*, 5941–5949.

Chang, Y. K., Silva, M. R., Gutkoski, L. C., Sebio, L., & Da Silva, M A. A. P. (1998). Development of extruded snacks using jatobá (*Hymenaea stigonocarpa* Mart) flour and cassava starch blends. *Journal of the Science of Food and Agriculture, 78*, 59–66.

Duke, J.A. & Wain, K.K. Medicinal Plants of the World. http://www.hort.purdue.edu/newcrop/duke_energy/Hymenaea_courbaril.html #Folk%20Medicine.

Henry, G. E., Momin, R. A., Nair, M. G., & Dewitt, D. L. (2002). Antioxidant and cyclooxygenase activities of fatty acids found in food. *Journal of Agricultural and Food Chemistry, 50*, 2231–2234.

Herlem, D., Huu, K.-F., & Kende, A. S. (1994). Studies toward the total synthesis of diterpenes in the labdane series III. Synthesis of two epimeric 6,7,8-trihydroxylabdadienes. *Tetrahedron, 50*, 2055–2070.

Imamura, P. M., Marsaioli, A. J., Barata, L. E. S., & Ruveda, E. A. (1977). ^{13}C NMR spectral analysis of eperuane diterpenes. *Phytochemistry, 16*, 1842–1844.

Jayaprakasam, B., Strasburg, G. A., & Nair, M. G. (2004). Potent lipid peroxidation inhibitors from *Withania somnfera* fruits. *Tetrahedron, 60*, 3109–3121.

Jayaprakasam, B., Zhang, Y., & Nair, M. G. (2004). Tumor cell proliferation and cyclooxygenase enzyme inhibitory compounds in Amaranthus tricolor. *Journal of Agricultural and Food Chemistry, 52*, 6939–6943.

Karl-Heinz, W., & Ibrahim, E. (2003). Biological relevance of terpenoids: overview focusing on mono, di and tetraterpenes. *Annals of Nutrition & Metabolism, 47*, 95–106.

Khoo, S. F., & Oehlschlager, A. C. (1973). Structure and stereochemistry of the diterpenes of *Hymenaea courbaril* (Caesalpinioideae) seed pod resin. *Tetrahedron, 29*, 3379–3388.

Krebs, H. C., Rakotoarimanga, J. V., & Habermehl, G. G. (1990). Isolation of spatulenol and (−)-caryophyllene oxide from *Vernonia mollissima* don and ^{1}H and ^{13}C reassignment by two-dimensional NMR spectroscopy. *Nuclear Magnetic Resonance in Chemistry, 28*, 124–128.

Lee, Y. T., & Langenheim, J. H. (1975). *Systematics of the genus Hymenaea* L. (Leguminosae, Caesalpinioideae, Detarieae) (Vol. 69). Chemistry of California Press Ltd., pp. 1–109.

Little, E. L. & Wadsworth, F. H. (1964). Common trees of Puerto Rico and the Virgin Islands. Ag. Handbook 249, USDA, Washington, DC.

Marsaioli, A. J., Leitao Filho, H., & De Paiva Campello, J. (1975). Diterpenes in the bark of *Hymenaea coubaril*. *Phytochemistry, 14*, 1882–1883.

Mitchell, J. A., & Warner, T. D. (1999). Cyclooxygease-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. *British Journal of Pharmacology, 128*, 1121–1132.

Mukherjee, D., Nissen, S. E., & Topol, E. J. (2001). Risk of cardiovascular events associated with selective COX-2 inhibitors. *Journal of American Medical Association, 286*, 954–959.

Nogueira, R. T., Shephered, G. J., Laverde, A., Jr., Marsaioli, A. J., & Imamura, P. M. (2001). Clerodane-type diterpenes from the seed pods of *Hymenaea courbaril* var. *stilbocarpa*. *Phytochemistry, 58*, 1153–1157.

Sivarajah, K., Lasker, J. M., & Eling, T. E. (1981). Prostaglandin synthetase-dependent cooxidation of (±)-benzo(*a*)pyrene-7,8-dihydrodiol by human lung and other mammalian tissues. *Cancer Research, 41*, 1834–1839.

Smith, W. L., DeWitt, D. L., & Garavito, R. M. (2000). Cyclooxygenases: structural, cellular, and molecular biology. *Annual Reviews of Biochemistry, 69*, 145–182.

Surh, Y.-J., Chun, K.-S., Cha, H.-H., Han, S. S., Keum, Y.-S., Park, K.-K., et al. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-κB activation. *Mutation Research, 480–481*, 243–268, and references cited therein.

Teramoto, T., Yuno, T., Morita, H., Katsumura, S., Sakaguchi, K., & Isoe, S. (1996). Synthesis of (±)-crotomachlin. *Synlett*, 141–142.

Witsberger, D., Current, D., & Archer, E. (1982). Arboles del Parque Deininger. Ministerio de Educación, El Salvador, pp. 146–147.

Zhang, Y., DeWitt, D. L., Murugesan, S., & Nair, M. G. (2005). Cyclooxygenase-2 enzyme inhibitory triterpenoids from Picrorhiza kurroa seeds. *Life Sciences, 27*, 3222–3230.