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Non-pungent functional food components in the water extracts of hot peppers

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ABSTRACT

Bhut Jolokia, the hottest pepper in the world, Scotch Bonnet and Jalapeño are hot *Capsicum* spp. with 1001,304, 250,000–300,000 and 5000 Scoville Heat Units (SHUs), respectively. The SHU is also an indication of varying concentrations of lipid-soluble capsaicinoids present in these hot peppers. However, our studies using lipid peroxidation (LPO) and cyclooxygenase (COX-1 and -2) enzyme assay with the water extracts of these peppers showed similarity in inhibitory activities. Also, chromatographic analyses of water extracts of these hot peppers showed identical profiles except for the capsaicinoids. This prompted us to investigate the water-soluble compounds in these pepper extracts with LPO and COX enzyme inhibitory activities. A bioassay-guided investigation of the water extract of Scotch Bonnet pepper afforded compounds **1**–**6**, also common in Jalapeño and Bhut Jolokia water extracts. Compounds **1–6** inhibited LPO by 73%, 45%, 20%, 43%, 16% and 72%, respectively, and COX-1 and -2 enzymes by 14–72% and 30–31%. Most of these compounds showed higher COX-1 inhibitory activity similar to aspirin, ibuprofen and naproxen. However, compound **5** showed higher COX-2 enzyme inhibition similar to Celebrex, a prescription non-steroidal anti-inflammatory (NSAID) drug. Compound **1** is novel; **2**, **3**, **5** and **6** were isolated from genus *Capsicum* for the first time.

1. Introduction

The genus *Capsicum* (Solanaceae) consists of a number of species, ranging from the very hot *frutescens*/*chinense* to moderately hot *annuum*, and each of these species further represent a number of varieties. The sweet and moderately hot peppers, including Jalapeño and chili, belonging to *Capsicum annuum*, are major crops in the USA. Also, the popularity of hot peppers is rapidly increasing amongst consumers. This is partly due to the diversification of food habits to include ethnic foods and the influence of the immigrant population throughout the nation. The heat principles in hot peppers are capsaicin and its analogues. Products containing capsaicin (Zostrix topical ointment) or patches are sold as topical pain relievers in the market in the USA and other countries. The proposed mechanism of action for this pain relief is based on the ability of capsaicin to inhibit the pain messenger "substance P". Although chili and other hot peppers are ingredients in everyday meals and processed foods, there is little information available on the water-soluble and non-pungent principles of these *Capsicum* spp. and their related health benefits when ingested as a food.

Capsaicinoids are considered as the major bioactive components in hot peppers (Krinsky, 2001; Marin, Ferreree, Tomas-Barberan, & Gil, 2004; Matsufuji, Matsufuji, Nakamura, & Takeda, 1998; Palevitch, Palevitch, & Craker, 1995). The heat values of Jalapeño, Scotch Bonnet and Bhut Jolokia are 5000, 250,000–300,000 and 1001,304 Scoville Heat Units (SHU), respectively (Bosland & Baral, 2007; Rowland, Villalon, & Burns, 1983; Yao, Nair, & Chandra, 1994). Of these three hot peppers, Jalapeño (*Capsicum annuum*, J) is a commonly used mild hot pepper in the USA. Scotch Bonnet (*Capsicum chinense*, SB), grown widely in Jamaica, is also presently being grown in the USA. The hottest chili pepper in the world, Bhut Jolokia (*Capsicum chinense*/*Capsicum frutescens,* BJ), originating from India, is now grown in Bangladesh, Sri Lanka and USA. We selected these peppers for our study based on consumer popularity and heat index of low (Jalapeño), medium (Scotch Bonnet) and high (Bhut Jolokia). Hot peppers are synonymous with capsaicin and the notion that capsaicinoids are the major or only bioactive compounds present in hot pepper. Therefore, we studied the non-pungent bioactive water-soluble compounds present in these hot peppers. In this paper we report LPO and COX-1 and COX-2 enzyme inhibitory glycosylated forms of glycerol, hydroxylated fatty acid and capsianoside for the first time from the aqueous-extracts of hot peppers.

2. Materials and methods

2.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on an INOVA Varian VRX 500 MHz instrument (Varian, Palo Alto, CA, USA). Merck silica gel 60

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(35–70 μM) was used for medium-pressure liquid chromatography (MPLC). The thin layer chromatography plates (20 × 20, 500 μm) were purchased from Analtech Inc. (Newark, DE, USA). Solvents (ACS grade) were used for isolation and purification of compounds. The COX-1 enzyme was prepared from ram seminal vesicles purchased from Oxford Biomedical Research, Inc. (Oxford, MI, USA). COX-2 enzyme was prepared from insect cells cloned with human PGHS-2 (prostaglandin endoperoxide H synthase-2) enzyme. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), aspirin, and 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were purchased from Sigma–Aldrich Chemical Company Co. (St. Louis, MO, USA). Vioxx®, Celebrex® and Bextra® were provided by Dr. S. Gupta, Sparrow Hospital, Michigan. 1-Stearoyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine (SLPC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). The fluorescent probe, 3-(p-(6-phenyl)-1,3,5-hexatrienyl) phenylpropionic acid was purchased from Molecular Probes (Eugene, OR, USA).

2.2. Plant material

Jalapeño, Scotch Bonnet and Bhut Jolokia peppers were harvested from plants grown in the greenhouses at Michigan State University. The seedlings were transplanted to 1-gallon pots containing sterile potting medium. The plants were watered with fertilizer (20:20:20, NPK) every two weeks and kept at 12-h photoperiod at 80 °F. All three peppers were planted and harvested at the same time under identical conditions to those stated above. Bhut Jolokia and Scotch Bonnet peppers were harvested when they were red and orange, respectively. Jalapeño pepper was harvested when it was about to turn red.

2.3. Extraction, chromatographic evaluation and preliminary bioassay of water extracts

Freshly harvested Bhut Jolokia, Scotch Bonnet and Jalapeño (150 g each) were blended separately with water (1000 ml × 3), centrifuged for 10 min and the supernatant lyophilised to afford water extracts weighing 12.3, 12.6 and 11.4 g, respectively. TLC analysis was carried out on silica gel plates using $CHCl_3$:MeOH (5:1) as the mobile phase. The HPLC analyses of water extracts of hot peppers were carried out on Xterra RP_{18} analytical column (5 μm, 4.6 × 250 mm; Waters Associates, Milford, MA, USA) and monitored at 280 nm using the mobile phase methanol:water (65:35, 0.8 ml/min) under isocratic conditions. Both TLC and HPLC analyses revealed that all three extracts contained identical components. Similarly, LPO and COX assay results showed similar activity profiles for all three extracts (Figs. 2 and 3). We used Scotch Bonnet water extract for further isolation and characterisation of water-soluble compounds in hot pepper extracts, although any one of the three extracts could have been used for this purpose.

2.4. Isolation of compounds *1*–*6*

An aliquot of Scotch Bonnet water-extract (2.5 g) was dissolved in MeOH, filtered and then subjected to C-18 column chromatography using gradient mobile phase (MeOH:H_2O, 5–100%, run time: 1.5 h; flow rate: 10 ml/min) to yield Fr. **1**, 200 ml; Fr. **2**, 160 ml; Fr. **3**, 170 ml; Fr. **4,** 170 ml; and Fr. **5**, 200 ml. Fr. **1**–**5** were evaporated separately under reduced pressure to remove the methanol and then the residue was lyophilised to afford dry powders 990, 210, 490, 126, and 395 mg, respectively. The compound in Fr. **1** was determined to be glucose, based on TLC profiles and NMR spectral data. The Fr. **2** was further purified by using PTLC [$CHCl_3$:MeOH (3:1)] to yield compound **5** (30 mg). Purification of Fr. **3** by PTLC [$CHCl_3$:MeOH (6:1)] gave compounds **3** (4.8 mg) and **4** (2.8 mg). The Fr. **4** was purified by using PTLC [$CHCl_3$:MeOH (10:1)] to afford compound **2** (4.2 mg). Similarly, the purification of Fr. **5** on silica gel plates by using [$CHCl_3$:MeOH (20:1)] as the mobile phase gave compounds **6** (12 mg) and **1** (28 mg).

2.4.1. Compound *1*

White solid (negative ion) HRESIMS *m/z*: 409.2948 $[M-H]^-$ (409.2954, calcd for $C_{23}H_{41}O_5$). ESI-MS: 409.3 $[M-H]^-$. UV λ_{max}: 230 nm. ^1H NMR (500 MHz, in $CDCl_3$): δ 5.31 (4H, m, H-11, 12, 14 and 15), 5.22 (1H, m, H-16), 4.26 (1H, dd, J = 4.5, 12.0 Hz, H-17a), 4.11 (1H, dd, J = 4.4, 11.5 Hz, H-17b), 2.73 (2H, m, H-13), 2.28 (4H, m, H-2 and 2′), 2.03 (2H, m, H-10), 1.59 (2H, m,), 1.28 (18H, m), 0.84 (6H, t, J = 7.0 Hz, Me-7′). ^{13}C NMR (125 MHz, in $CDCl_3$): δ 173.2, 172.8 (C-1 and 1′), 130.3, 130.1, 128.3, 128.1 (C-11, 12, 14 and 15), 69.1 (C-17), 62.2 (C-16), 34.3 (C-13), 34.2, 34.2, 32.1, 31.7, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 27.4, 25.8, 25.1, 22.9 (overlapped CH_2), 14.3 (Me-7′). The identity of compound **1** was confirmed as shown in Fig. 1.

2.4.2. Compound *2*

White powder. ^1H NMR (500 MHz, in CD_3OD) δ: 1.37, 1.63 × 2, 1.77 (each 3H, s, Me-20, 19, 16), 1.97–2.29 (10H, m, H-5, 8, 9, 12, 13), 4.15, 4.33 (each 1H, d, J = 10.0 Hz, H-17), 5.12 (2H, t, J = 8.0 Hz, H-6, 10), 5.06 (1H, d, J = 10.0 Hz, H-1), 5.21 (1H, d,

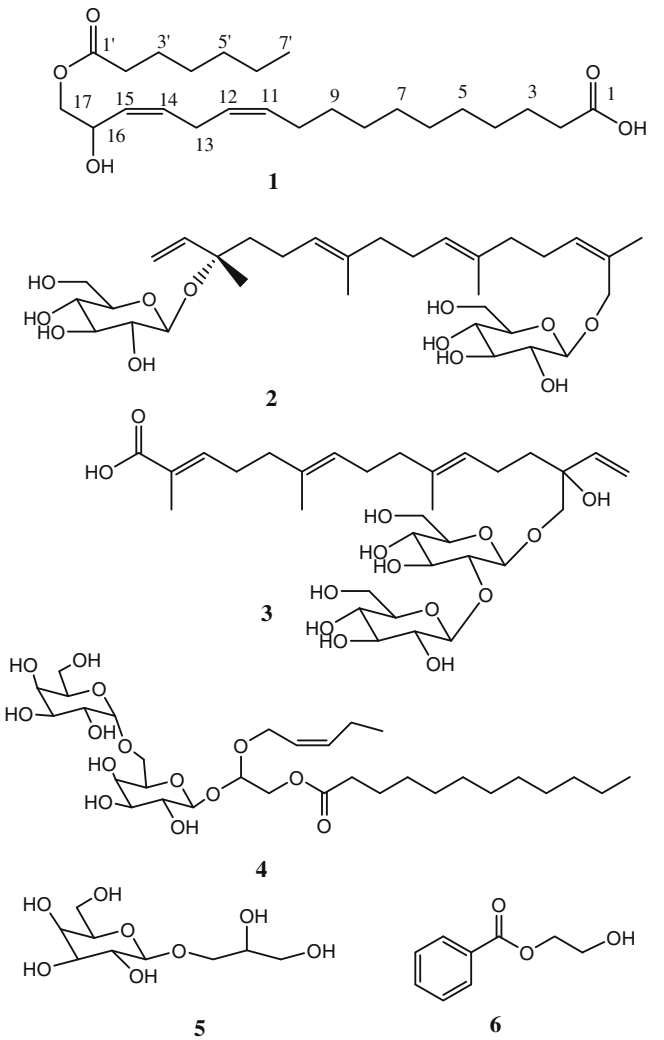

Fig. 1. Structures of compounds **1**–**6** isolated from the water extract of Scotch Bonnet pepper.