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Original Communication

Antihypertensive, antiinflammatory and antioxidant activities of breadfruit leaf tea

Keisha R. Christian[1], Shilpa Pothu[1], Muraleedharan G. Nair[2], Priyanka Prathipati[3], Keith E. Jackson[3] and Omar E. Christian[1,*]

[1]Department of Chemistry and Physics, McNeese State University, Box 90455, Lake Charles LA 70609; [2]Bioactive Natural Products and Phytoceuticals, Department of Horticulture and National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824; [3]Department of Basic Pharmaceutical Sciences, University of Louisiana, Monroe, Bienville 370, 1800 Bienville Drive, Monroe, LA 71201, USA.

ABSTRACT

The brewed tea extracts of the green and yellow breadfruit leaves were evaluated for their antihypertensive potential using Sprague-Dawley rats. There was significant attenuation (20-30 mm Hg) of the angiotensin II-mediated hypertension in the animals treated with the tea extracts. The antiinflammatory and antioxidant activities of these extracts were determined. The antioxidant activities of the brewed whole green leaf and yellow leaf teas, measured by fluorescence spectroscopy using a model liposome system, were 90.2% and 80.5%, respectively. The yellow and green leaf whole teas displayed moderate cyclooxygenase (COX)-1 and -2 enzyme inhibitory activities.

KEYWORDS: breadfruit *Artocarpus communis*, antihypertensive, antiinflammatory, antioxidant

INTRODUCTION

Hypertension is often referred to as a 'silent killer', as it can remain asymptomatic for many years, manifesting later in life as a myriad of other diseases. Cardiovascular diseases, diabetes, peripheral artery disease, cerebrovascular diseases and kidney failure are some of the illnesses associated with hypertension [1, 2]. Well-defined links exist among the modulation of inflammation, oxidative stress and hypertension [3, 4]. The inflammatory response results in the release of prostaglandins. These intermediates are known to modulate the onset and progression of hypertension. Current literature suggests that oxidative stress results in the increased production of reactive oxygen species which has been shown to decrease nitric oxide production leading to vasoconstriction, a root cause of hypertension [5].

The consumption of foods rich in flavonoids reduces the overall risk of hypertension and other cardiovascular diseases [6]. *Artocarpus communis* (breadfruit) is widely consumed in the Caribbean and South Pacific Islands [7, 8]. A decoction of its leaves is used as a folk-medicinal therapy for hypertension in several Caribbean countries. Preliminary reports have indicated that the methanolic extracts of *A. communis* exerted a negative inotropic effect in mice and significantly reduced the left ventricular pressure *in vivo* [9]. A more recent *in vitro* study confirmed that the organic leaf extracts of *A. communis* exhibited antihypertensive properties by inhibiting the well-known mediator, angiotensin-converting enzyme [10]. However, the metabolites responsible for the observed antihypertensive activity in either report were not identified. There is a plethora of data on prenylated flavonoids, which exhibit antiinflammatory [11] and antioxidant [12] activities.

*Corresponding author: ochristian@mcneese.edu

MATERIALS AND METHODS

General experimental procedure

Mass spectral analysis was performed using a Varian 320 triple quadropole mass spectrometer, fitted with an electrospray ionization (ESI) source operated in positive mode. The capillary voltage was set at 70 eV with a nebulizing gas flow rate of 12 L/hr, and the drying gas temperature was set at 300 ^{o}C. The samples (20 µL) were injected and separation was performed on a Kinetex C_{18} column (100 mm x 4.6 mm, 2.6 µm).

Plant material

The leaves of *A. communis* were collected from the Agricultural Experimental Stations at the University of the Virgin Islands campus (07VI002) and in Dorothea (07VI003), St. Thomas, Virgin Islands.

Extraction and isolation

The green and yellow leaves of *A. communis* (2 kg each) were cut into small pieces and brewed using distilled water (2 L) and left overnight. The whole tea extracts, green breadfruit leaf whole tea (GBLT), 340 mg and yellow breadfruit leaf whole tea (YBLT), 370 mg, were then partitioned with ethyl acetate (3 x 100 mL). The organic layers were reduced *in vacuo* to afford the green breadfruit leaf tea ethyl acetate extract (GBLTE), 126 mg and the yellow breadfruit leaf tea ethyl acetate extract (YBLTE), 146 mg. The remaining aqueous portions were reduced *in vacuo* to yield the green breadfruit leaf tea aqueous extract (GBLTAq), 180 mg and the yellow breadfruit leaf tea aqueous extract (YBLTAq), 205 mg.

Antihypertensive assay

Male Sprague-Dawley rats (250-350 g; n = 12, Harlan, Indianapolis, IN) were used in this study. This protocol was approved by the University of Louisiana at Monroe Institutional Animal Care and Use Committee. Prior to the experiments, the rats were housed in a controlled environment and had free access to commercial rat chow and tap water. Rats were anesthetized with a single injection of thiobutabarbital sodium (120 mg/kg, intraperitoneal) and a tracheal tube was inserted to maintain an open airway. Catheters (PE-50 tubing filled with heparinized saline) were implanted into a carotid artery and a jugular vein to allow for continuous monitoring of mean arterial pressure (MAP)/heart rate and for intravenous administration of drugs, respectively. The arterial catheter was connected to a pressure transducer (model TSD104A, Biopac Systems, Santa Barbara, CA) and the venous catheter was connected to a Sage micro infusion pump (Orion Research, Inc., model M361, Boston, MA) set at a 1 mL/hour saline infusion rate. The rats were treated with angiotensin II (7 ng/kg/min). A bladder cannula was inserted to allow urine collection for the determination of urinary volume and urinary concentrations of sodium/potassium. Following the surgical procedures, the rats were allowed to stabilize for 15 min. After this initial stabilization period, 15 min of baseline data was collected and the animals were then treated with either vehicle (control) and angiotensin II or angiotensin II and brewed tea. The brewed tea was administered at a concentration of 1 mg/mL and at an infusion rate of 1 mL/hr.

Antioxidant activity screening assay

The antioxidant activity screening assay was conducted on the tea samples according to the previously reported method [13]. The fluorescence probe was prepared from 3 mg of 3-[p-(6-phenyl)-1,3,5-hexatrienyl]phenyl propionic acid in 3 mL dimethyl formamide (DMF), diluted 20 fold and stored at -50 °C. All samples were dissolved in dimethyl sulphoxide (DMSO) and assayed at a concentration of 250 ppm. Each sample was assayed in duplicate and the percentage inhibition was calculated with respect to DMSO. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tetrabutyl hydroquinone (TBHQ) were used as the controls at a concentration of 1 ppm. Fluorescence was measured at 384 nm and monitored at 0, 1, 3 min and every 3 min thereafter up to 21 min using a Turner Model 450 Digital Fluorometer (Barnstead Thermolyne, Dubuque, IA). Relative fluorescence was calculated by dividing a given fluorescence by the fluorescence at time = 0 min.

Cyclooxygenase inhibitory activity screening assay

The cyclooxygenase-1 enzyme inhibitory assay was conducted using an enzyme preparation from

ram seminal vesicles and COX-2 inhibitory activity was determined using a preparation from insect lysate [13]. Both the enzymes were stored at -80 °C until ready for use. All the tea samples were dissolved in DMSO and assayed at a concentration of 250 µg/mL. Standard COX inhibitors such as Aspirin (60 µM), Celebrex (26 nM) and Vioxx (32 nM) were used as positive controls. Each sample was assayed in duplicate and the percentage inhibition was calculated with respect to DMSO. The percent COX-1 and COX-2 inhibition was calculated relative to the control solvent.

RESULTS AND DISCUSSION

Ethnomedicinal claims remain an important lead in the identification of biologically active natural products. Spurred on by several such claims, we investigated the antihypertensive properties of tea prepared from the leaves of *A. communis*. Throughout the Caribbean, the yellow leaves are usually brewed and consumed as a tea to lower blood pressure. In our ongoing effort to identify new antihypertensive metabolites from tropical plants, the whole tea extract GBLT and YBLT of the breadfruit plant were examined. The leaf decoction was assayed for antihypertensive activity using a rat model.

A subset of the rats was treated with the tea extract. The extracts were administered, separately, by intravenous injections at concentrations of 1 mg/mL with an infusion rate of 1 mL/hr for 45 min. The samples were run in duplicate (GBLT/YBLT 1 and 2). There was a significant reduction in mean arterial pressure (MAP) (20-30 mmHg) in animals that were given both GBLT and YBLT (Figure 1).

The angiotensin II inhibitory activity observed in the tea extracts is not surprising given the rich diversity of antiinflammatory prenylated flavanoids identified from *A. communis* [14-17]. Flavonoids, in particular flavones, have been recognized for their moderate angiotensin-converting enzyme inhibitory potential [18, 19]. The C2-C3 double bond present in the flavone class of metabolites is identified as one of the main structural features contributing to the angiotensin-converting enzyme inhibition [18]. In this study we identified flavone 14-hydroxyartonin E (**1**) as the major constituent of the brewed tea extracts of both GBLT and YBLT. This compound was previously identified from *Artocarpus lanceifolius* [19].

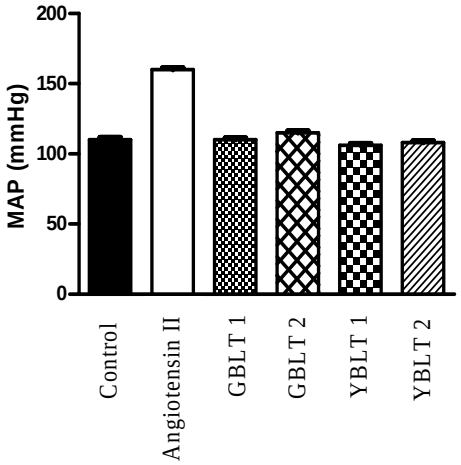
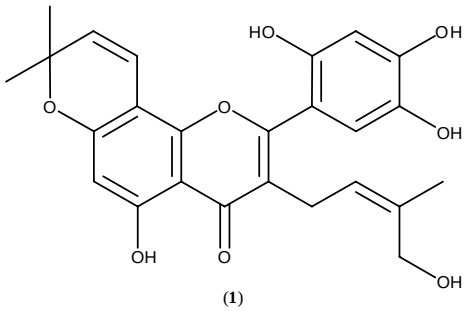


Figure 1. The attenuation of the angiotensin II-mediated hypertension with breadfruit leaf extract. GBLT (Green breadfruit leaf tea whole extract), YBLT (Yellow breadfruit leaf tea whole extract).



(**1**)

Recognizing the association between the angiotensin-converting enzyme (ACE) inhibition and the inflammatory response [20], we screened our extracts for COX-2 activity. The YBLT, GBLT and YBLTE displayed similar COX-2 enzyme inhibitory activities of 11%, 13% and 13%, respectively (Figure 2). The GBLTAq and YBLTAq showed marginally selective COX-1 inhibition of 12% and 5%, respectively. The data indicates that the antiinflammatory metabolites were concentrated in the non-polar organic extract of the tea; the more polar constituents that reside in the remaining aqueous tea extract preferentially inhibited the COX-1 enzymes.

Prenylated flavonoids isolated from the *Artocarpus* genus have shown significant antioxidant properties [21]. The antioxidant activity of the GBLT and YBLT were measured by fluorescence spectroscopy using a model liposome system. The data indicated a

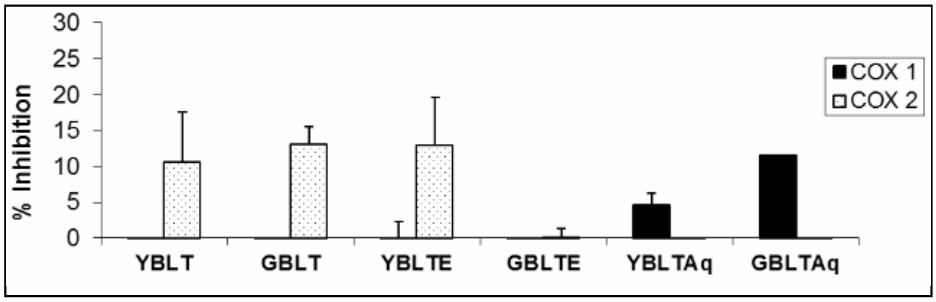


Figure 2. Cyclooxygenase inhibitory activities of the green and yellow breadfruit leaf tea. YBLT (Yellow breadfruit leaf whole tea); YBLTE (ethyl acetate extract of YBLT); YBLTAq (remaining aqueous extract of YBLT); GBLT (Green breadfruit leaf whole tea); GBLTE (ethyl acetate extract of GBLT); GBLTAq (remaining aqueous extract of GBLT).

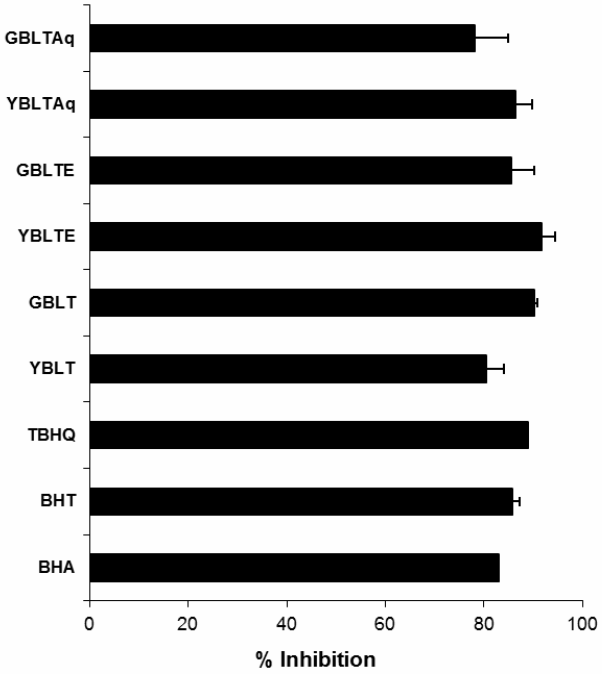


Figure 3. Antioxidant activity of the green and yellow breadfruit leaf tea. YBLT (Yellow breadfruit leaf whole tea); YBLTE (ethyl acetate extract of YBLT); YBLTAq (remaining aqueous extract of YBLT); GBLT (Green breadfruit leaf whole tea); GBLTE (ethyl acetate extract of GBLT); GBLTAq (remaining aqueous extract of GBLT).

relatively high antioxidant activity for both whole tea extracts GBLT and YBLT with 90.2% and 80.5% inhibition, respectively (Figure 3). The organic extracts YBLTE and GBLTE displayed a 91.7% and 85.4% inhibition, respectively in the liposomal model. The aqueous extracts of both teas, YBLTAq and GBLTAq displayed inhibitory activity of 86.3% and 78.0%.

CONCLUSION

This is the first *in vivo* study of the bioactivity of the brewed tea of breadfruit leaves. The observed antihypertensive activity of the whole tea extracts of breadfruit leaves could be due to one of the previously known flavonoids, particularly due to their antioxidant and antiinflammatory activities. Additional work is needed to further elucidate the structures of potentially active compounds and to quantify their amounts in the yellow bread fruit tea. This information will help to determine the effective dose of daily consumption of this tea for achieving its acclaimed health benefits.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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