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# Anti-inflammatory and antioxidant activities of *Coccoloba uvifera* (Seagrapes)

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## ABSTRACT

Seagrapes (*Coccoloba uvifera*) are widely consumed in Florida and the Caribbean. The edible berries are anecdotally reported to have antihypertensive and anti-asthmatic properties. The intense red/purple color of the ripe fruits indicates the presence of components containing chromophores with extended conjugation; these may potentially include anthocyanins or other polyphenolics. The crude seagrape berry extracts displayed significant anti-inflammatory and antioxidant activity *in vitro* at a concentration of 250 µg/mL. The crude methanolic extract displayed the COX-2 selectivity similar to the commercial standard Vioxx. The inhibition of lipid peroxidation of the hexane, ethyl acetate and methanolic extracts were 80%, 45%, and 77% respectively. The antioxidant activities of the three extracts were comparable to the commercially available antioxidants, butylated hydroxytoluene, butylated hydroxyanisole and tert-butylhydro-quinone. Cyanidin (**1a**), delphinidin (**2a**), malvidin (**3a**) and petunidin (**4a**) were identified from the methanolic extract by LCMS/MS analysis. This is the first report of these metabolites from *Coccoloba uvifera* (seagrapes).

**KEYWORDS:** seagrapes, *Coccoloba uvifera*, anthocyanins

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## INTRODUCTION

Diets rich in fruits and vegetables have been implicated in lowering an individual's overall risk of certain cardiovascular and other diseases triggered by inflammation and oxidative damage. Recently, "super fruits" like acai and pomegranate have been the focus of intense scientific scrutiny due to their anti-inflammatory and antioxidant activities resulting, in large part, from the presence of various polyphenolic components, including anthocyanins [1].

*Coccoloba uvifera* (Polygonaceae), commonly known as seagrapes, is widely consumed in Florida and the Caribbean. The plant is very tolerant to coastal conditions, thriving in sandy well-drained soil, and is used in landscaping as well as for protecting beaches from erosion. The edible berries are anecdotally reported to have anti-hypertensive and anti-asthmatic properties, and are eaten fresh or processed into jams, jellies or other preserves. The intense red/purple color of the ripe fruits indicates the presence of components containing chromophores with extended conjugation; these may potentially include anthocyanins or other polyphenolics. Despite the rich folk medicinal heritage that surrounds this plant, and its wide consumption, there are virtually no phytochemical data on its edible berries.

The lone publication on the chemistry of the seagrape fruits examined the volatile constituents

via gas chromatography [2]. Ascorbic acid, as well as 32 other volatile constituents, and cyclopentylacetic acid were identified. The other substantive publication in this area focused on the antioxidant and anti-tyrosinase activity of the root extract [3]. This root extracts inhibited the over expression of melanocyte-stimulating hormone, which is implicated in photooxidative tissue damage and hyperpigmentation. These data support the use of root extracts in cosmetics. The presence of antioxidant metabolites in the root extract of seagrapes is not surprising, as the family (Polygonaceae) is well known to harbor several species whose metabolites display significant anti-inflammatory and antioxidant activities. It is with the bioactivities of these metabolites in mind, that we started to examine the potential benefits of consuming the fruits of *C. uvifera*.

As a part of our on-going study of phytoceuticals from commonly consumed Caribbean plants, we herein describe the anti-inflammatory and antioxidant activity of the methanolic extract of *C. uvifera*. Four known anthocyanidins delphinidin (**1a**), cyanidin (**2a**), malvidin (**3a**) and petunidin (**4a**) were identified from the methanolic extract.

## EXPERIMENTAL

### General experimental

LCMS analysis was performed using a Varian 320 MS series liquid chromatography/mass selective detector equipped with a triple quadropole mass detector. The liquid chromatography system consisted of two binary pumps, an in-line degasser and a manual injector connected to the mass spectrometer. The samples (20 µL) were injected into the HPLC and separation was performed on a Polaris C18 reverse phase column (100 x 4.6 mm ID, 5 µm). Gradients of 0.1% formic acid in deionized water (solvent A) to 0.1% formic acid in acetonitrile (solvent B) were used to elute the columns. The flow rate was set at 400 µL/min, the MS was 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, the drying gas temperature was set at 300$^{o}$C. Mass spectral data were acquired in scan mode (*m/z* 200 - 1000).

### Extraction and isolation

*Coccoloba uvifera* fruits were collected along the Brewers Bay beach in St Thomas US Virgin Islands. The seeds were removed and the fruit flesh (2 kg) sequentially extracted with acidified methanol (0.1% formic acid, 4 x 250 mL), ethyl acetate (4 x 250 mL) and hexane (4 x 250 mL). The bright red methanolic extract (500 g) was stored at -20$^{o}$C.

### Total phenolic assay

The total phenolic composition of the methanol extract of *C. uvifera* fruits was determined with the Folin-Ciocalteu method using gallic acid as a standard as outlined by Singleton *et al.* [4]. Results were expressed as milligrams of gallic acid per gram of extract.

### Antioxidant activity screening assay

The antioxidant activity screening assay was conducted according to the method of Christian, K. *et al.* [5]. The fluorescence probe was prepared from 3 mg of 3-[p-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (DPH-PA) in 3 mL DMF diluted 20 fold and stored at -50˚C. All samples were dissolved in DMSO and assayed at a concentration of 250 µg/mL. Each sample was assayed in triplicate and the percentage inhibition was calculated with respect to DMSO. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydro-quinone (TBHQ) were used as the positive control at a concentration of 1 µg/mL. 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 (COX) activity screening assay

The COX-1 enzyme inhibitory assay was conducted using an enzyme preparation from ram seminal vesicles and the COX-2 inhibitory activity was determined using a preparation from insect lysate according to the method by Christian, K. *et al.* [5]. Both enzymes were stored at -80˚C until ready for use. All samples were dissolved in DMSO and assayed at a concentration of 250 µg/mL. Standard COX inhibitors Aspirin (60 µM), Celebrex (26 nM) and Vioxx (32 nM) were used as positive controls. Each sample was assayed in triplicate and the percentage inhibition was calculated with respect to DMSO.

The percent COX-1 and COX-2 inhibition was calculated relative to the blank using the following formula:

% COX Inhibition = $(RO_s - RO_b)/ RO_b$ x 100

Where $RO_s$ was the rate of oxygen uptake for the samples and $RO_b$ was the rate of oxygen uptake for the DMSO blank.

## RESULTS AND DISCUSSION

Oxidative damage from reactive oxygen species (ROS) is widely accepted as one of the contributing factors to tumorgenesis [6]. Fruits rich in anthocyanins and other polyphenolics are known to scavenge ROS and are therefore beneficial [7]. The percentage inhibition of peroxidation, 80%, 40%, and 80%, for the hexane, ethyl acetate and methanolic extracts, respectively, were significant compared to the known standards, BHA, BHT and TBHQ, Figure 1 ($P$<0.05). This activity is not surprising as the seagrape berries are suspected to contain several anthocyanins and polyphenolics. Furthermore, the total phenolic content of the methanolic extract was 3.2 x $10^{-1}$ mg gallic acid equivalence per gram of extract.

The methanol extract displayed marked selectivity, inhibiting only COX-2 (29 %) at a concentration of 250 µg/mL, Figure 2 ($P$<0.05). The selectivity observed in the crude extracts suggest that there may be other metabolites contributing to the activity as anthocyanins usually exhibit only moderate selectivity for the COX 2 isoform [8]. The ethyl acetate and hexane extracts inhibited both COX-1 and COX-2 enzymes, 6% and 20% for the ethyl acetate extract, 20% and 22% for the hexane extract. The selectivity observed for the methanolic extract is similar to the activity observed for Vioxx. The COX-2 isoform has been implicated in several inflammatory diseases like asthma and arteriosclerosis [9], and more recently cancer [10]. The study of how the current therapies may be augmented with a combined COX-2 inhibitor dosage regiment may be beneficial. These data support the use of seagrape berries as a folk medicinal treatment for asthma and supports its use as an anti-inflammatory agent.

The LCMS/MS profile of the methanolic extract revealed the presence of five anthocyanidins (Table 1). Cyanidin (**1a**) and delphinidin (**2a**) were the most abundant based on the relative peak heights of the ion chromatogram. Malvidin (**3a**) and petunidin (**4a**) were also present in minor quantities. The anthocyanins identified are present as either the glucoside (**1b** - **4b**) or the galactoside

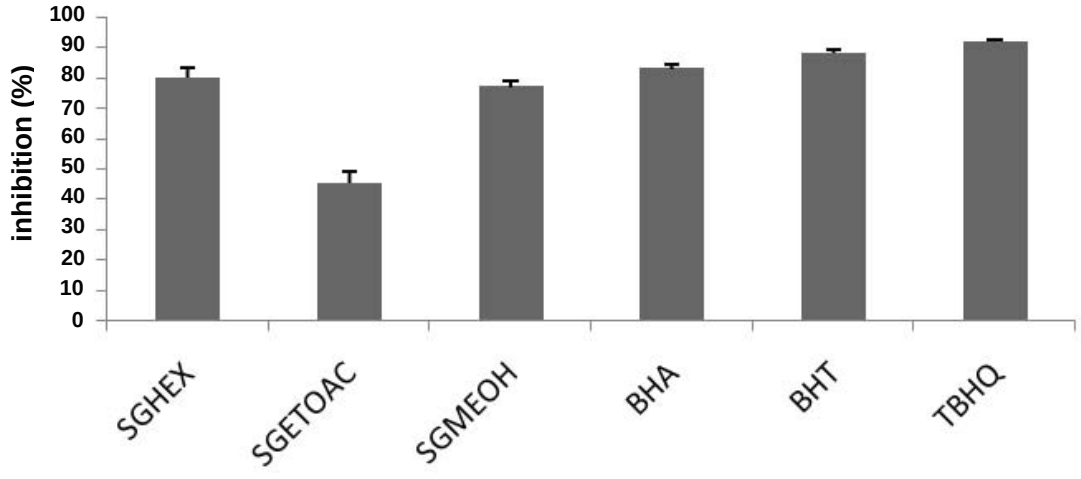



**Figure 1.** Antioxidant activity of the hexane (SGHEX), ethyl acetate (SGETOAC) and methanol (SGMEOH) extracts of seagrapes. Extracts were tested at 250 µg/mL. The percent inhibition was calculated with respect to solvent control and the values represent the mean ± SD. DMSO was used as the solvent control. The positive controls BHA, BHT and TBHQ were tested at 1 µg/mL.

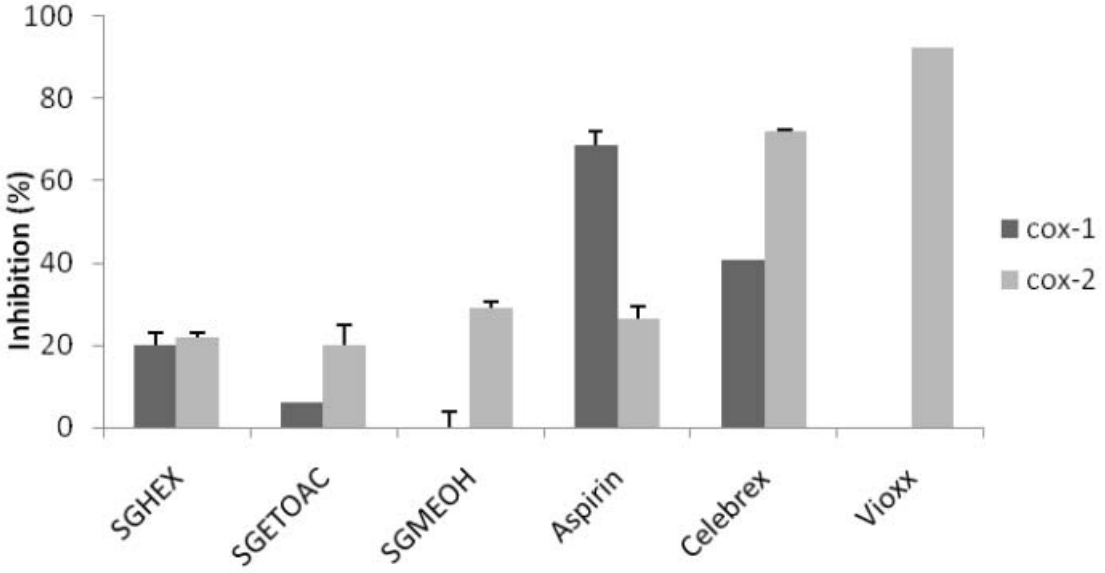



**Figure 2.** Cyclooxygenase inhibitory activity of the hexane (SGHEX), ethyl acetate (SGETOAC), and methanol (SGMEOH) extracts of seagrapes. Extracts were tested at 250 μg/mL. The percent inhibition was calculated with respect to solvent control and the values represent the mean ± SD. DMSO was used as the solvent control. The positive controls were the anti-inflammatory drugs Aspirin (60 μM), Celebrex (26 nM) and Vioxx (32 nM).

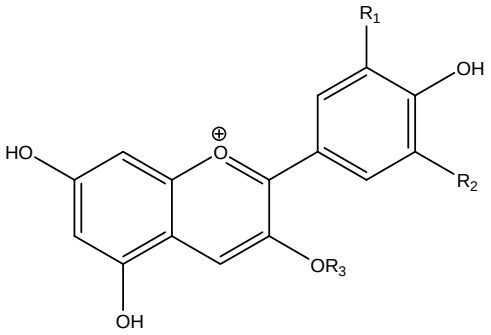
**Table 1.** LCMS/MS identification of anthocyanins in the methanolic extract of seagrapes.

| Anthocyanin | Mass of anthocyanin/aglycone | Formula (aglycone) |
|---|---|---|
| Cyanidin-3-glc/gal | 449/287 | $C_{15}H_{11}O_6^+$ |
| Delphinidin-3-glc/gal | 465/303 | $C_{15}H_{11}O_7^+$ |
| Delphinidin-3-ara | 435/303 | $C_{15}H_{11}O_7^+$ |
| Malvidin-3-glc/gal | 493/331 | $C_{17}H_{15}O_7^+$ |
| Petunidin-3-glc/gal | 479/317 | $C_{16}H_{13}O_7^+$ |

glc - glucoside, gal - galactoside, ara - arabinoside.

(**1b** - **4b**). Delephinidin-3-arabinoside (**2c**) was also observed in the methanolic extracts (Figure 3). Our preliminary DFT calculations suggest that the glucosides of cyanidin and delphinidin were energetically favored of the glucoside/galactoside pair, this data supports the previously observed trend [11].

This study represents the first phytochemical analysis of the fruits of *C. uvifera*. The anthocyanins identified may account for the antioxidant and anti-inflammatory activity in the methanolic extract. While the anthocyanidin content was not quantitatively determined, cyanidin (**1a**) and dephinindin (**2a**) are often the most abundant pigments observed in plants [12, 13]. The color of the ripe grapes is consistent with the major pigments observed [11]. Finally, the antioxidant and anti-inflammatory activities of the fruits suggest that the consumption of seagrapes can provide significant health benefits. The COX-2 selectivity is promising and suggests that diets rich in fruits [14] like seagrapes may help to combat inflammatory diseases including cardiovascular diseases.




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Cyanidin (**1a**) | OH | H | H |
| Cyanidin-3-glucoside (**1b**) | OH | H | glucose |
| Delphinidin (**2a**) | OH | OH | H |
| Delphinidin-3-glucoside (**2b**) | OH | OH | glucose |
| Delphinidin-3-arabinoside (**2c**) | OH | OH | arabinose |
| Malvidin (**3a**) | OMe | OMe | H |
| Malvidin-3-glucoside (**3b**) | OMe | OMe | glucose |
| Petunidin (**4a**) | OMe | OH | H |
| Petunidin-3-glucoside (**4b**) | OMe | OH | glucose |

**Figure 3.** Anthocyanins identified from seagrapes.

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