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# Polyphenolic Antioxidants from the Fruits of *Chrysophyllum cainito* L. (Star Apple)

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*Chrysophyllum cainito* L. (Sapotaceae), known commonly as star apple or caimito, is a tropical tree that bears edible fruits. The fruits are grown commercially in certain tropical and subtropical areas, such as southern Florida. In this study, the fresh fruits were extracted with methanol and partitioned with hexane and ethyl acetate sequentially. The ethyl acetate soluble fraction displayed high antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay ($IC_{50}$ = 22 $\mu$g/mL). Activity-guided fractionation of the ethyl acetate soluble fraction was performed to identify the antioxidant constituents. Nine known polyphenolic antioxidants, (+)-catechin (**1**), (−)-epicatechin (**2**), (+)-gallocatechin (**3**), (−)-epigallocatechin (**4**), quercetin (**5**), quercitrin (**6**), isoquercitrin (**7**), myricitrin (**8**), and gallic acid, have been identified from the fruits. Of these nine antioxidants, **2** is present in the highest concentration in star apple fruits (7.3 mg/kg fresh weight), and **5** showed the highest antioxidant activity ($IC_{50}$ = 40 $\mu$M) in the DPPH assay.

**KEYWORDS:** ***Chrysophyllum cainito*; Sapotaceae; star apple; caimito; antioxidants; polyphenols; (+)-catechin; (−)-epicatechin; (−)-epigallocatechin; gallic acid; (+)-gallocatechin; isoquercitrin; myricitrin; quercetin; quercitrin**

## INTRODUCTION

Plants contain a wide variety of chemicals that have potent antioxidant activity. The best-known phytochemical antioxidants are traditional nutrients, such as $\beta$-carotene, ascorbic acid, and α-tocopherol. However, there is growing evidence that a significant portion of the antioxidant capacity of many food plants is due to compounds other than the traditional vitamins (*1*). Epidemiological studies indicate that fruit and vegetable consumption is inversely related to cancer and coronary heart disease mortality, and some researchers have suggested that this reduction is not solely due to increased levels of vitamins and fibers (*2*, *3*). Other compounds, such as polyphenolics, appear to play an important role in the overall antioxidant capacity of fruits and vegetables.

*Chrysophyllum cainito*, known commonly as star apple (or caimito in Spanish), is a tree indigenous to Central America that grows 8−30 m tall. In the United States, it grows well only in the warmest locations in southern Florida (*4*). Star apple fruits are pear-shaped, 5−10 cm in diameter, and red-purple or pale green. When the fruit is cut in half, there is a distinctive star star-shaped array of eight segments. The flesh is smooth, tastes sweet, and is pleasantly aromatic. The fruit is typically eaten by cutting it in half and spooning out the flesh. The skin and the rind are inedible (*4*).

Although the fruits are considered to be very tasty, they are of minor commercial importance in the United States as compared to other Sapotaceae fruits (*4*). In southern Florida, for example, there are approximately six scattered acres of commercially grown and harvested *C. cainito* trees. Florida does not maintain economic data on this crop's worth, but the fruit is sold at farm gate for $1−3 per pound, and mature trees may bear 75−200 lbs of fruit per year (personal communication, Dr. Jonathan Crane, University of Florida, Tropical Research Station). The fruit is also grown commercially in Australia, and in August 1999, the wholesale price per 6 kg tray in Sydney was about $33.00 (Australian) (*5*).

A nutritional analysis of *C. cainito* fruit showed that a typical serving contains 67.2 calories, with 0.72−2.33 g of protein, 14.7 g of carbohydrates, and 0.55−3.30 g of fiber (*4*). The vitamins contained in star apple fruits include carotene (0.004−0.039 mg), thiamine (0.018−0.08 mg), riboflavin (0.013−0.04 mg), niacin (0.935−1.340 mg), and ascorbic acid (3.0−15.2 mg) (*4*). Levels of the amino acids tryptophan, methionine, and lysine as well as calcium and phosphorus have been reported for star apple fruits (*4*). Two compounds, $\beta$-amyrin acetate and gentisic acid, have been identified from the leaves of this species (*6*, *7*). We are not aware of any additional phytochemical studies on the constituents of *C. cainito*. In addition to its culinary uses, star apple fruit is also used in folk medicine for inflammation

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associated with laryngitis and pneumonia and for the treatment of diabetes mellitus (*4*). In eastern Nicaragua, the Garifuna use decoctions of the fruit to treat diarrhea, fever, and venereal disease and as an astringent (*8*).

We recently began to screen approximately 50 tropical fruits and vegetables for their antioxidant capacities (Kennelly, unpublished). The fresh fruits were extracted with MeOH and then partitioned sequentially with hexane and EtOAc. The EtOAc fraction produced in this manner does not contain the classical antioxidant vitamins, such as the lipid soluble $\beta$-carotene and $\alpha$-tocopherol and the water soluble vitamin C. We were therefore able to concentrate our investigation on polyphenolic antioxidants from the EtOAc fraction. The EtOAc fraction of *C. cainito* was found to be one of the most active ($IC_{50} = 22$ $\mu$g/mL) in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Therefore, as part of our program to identify cardioprotective compounds from edible plants, *C. cainito* was subjected to activity-guided fractionation. This report describes the isolation and identification of nine antioxidant constituents, (+)-catechin (**1**), (−)-epicatechin (**2**), (+)-gallocatechin (**3**), (−)-epigallocatechin (**4**), quercetin (**5**), quercitrin (**6**), isoquercitrin (**7**), myricitrin (**8**), and gallic acid, from *C. cainito*.

## MATERIALS AND METHODS

**Plant Material.** Fruits of *C. cainito* were collected from the Fruit and Spice Park (Homestead, FL) in April 2000. Frozen fruits were shipped to New York City by overnight courier and stored at −20 °C until extracted. A voucher specimen of *C. cainito* was prepared, identified, and deposited at the herbarium of The New York Botanical Garden (Bronx, NY).

**Extraction and Isolation Procedures.** The fresh frozen fruits (5.2 kg) of *C. cainito* were extracted with MeOH twice at room temperature. After the MeOH was removed in vacuo, additional $H_2O$ was added, and the resulting aqueous extract was partitioned with hexane and EtOAc, respectively. The EtOAc fraction ($IC_{50} = 22$ $\mu$g/mL) was concentrated in vacuo to give 8.8 g of a residue, of which 6.0 g was subjected to vacuum−liquid chromatography (VLC) over silica gel, eluting with gradient mixtures of $CHCl_3$/MeOH (from 100% $CHCl_3$ to 100% MeOH). The resulting fractions were examined by silica gel 60 and reversed-phase C18 (RP18) thin-layer chromatography (TLC), and those fractions with similar TLC profiles were combined to give a total of 13 fractions (A−M). All fractions were tested in the DPPH assay, and fractions F (175 mg), G (210 mg), H (230 mg), I (400 mg), and J (430 mg) were the most active ($IC_{50} < 50$ $\mu$g/mL) in the DPPH assay.

Fraction J was separated over Sephadex LH-20 (from 1:1 EtOH/$H_2O$ to 100% EtOH). Subfraction J-6 was purified repeatedly by RP18 column chromatography (CC) eluting with a gradient of 1:4 to 1:1 MeOH/$H_2O$ to yield three flavan-3-ols, **1**−**3**. Subfraction J-7 was purified by RP18 CC eluting with a gradient of 1:4 to 1:1 MeOH/$H_2O$ to obtain **4**. Fraction F was separated again over Sephadex LH-20 eluting with a solvent gradient of 3:2 EtOH/$H_2O$ to 100% EtOH to yield seven fractions. The last fraction, F-7, was recrystallized with $H_2O$ and MeOH to give pure **5**. Fraction H was separated by RP18 CC (gradient from 1:4 to 1:1 MeOH/$H_2O$), and the first fraction collected was identified as gallic acid. Fraction I was chromatographed again over Sephadex LH-20 (gradient from 1:1 EtOH/$H_2O$ to 100% EtOH) to give eight subfractions. Subfractions I-6 and I-7 were combined and chromatographed by preparative RP18 TLC (MeOH/$H_2O$) to yield **6** and **7** and **8** as a defined mixture. A total of eight flavonoids (**Figure 1**) and gallic acid were identified as antioxidant constituents of *C. cainito*. The properties of the compounds are presented below.

**(+)-Catechin (1).** The separation gave 9.2 mg of white powder; yield 2.5 mg/kg fresh weight. Negative electrospray ionization mass spectroscopy (ESIMS) *m/z* 289 $[M - 1]^-$. $^1H$ and $^{13}C$ nuclear magentic resonances (NMR) are consistent with previously published data (*9*, *10*). The identification was further supported by RP18-TLC (MeOH/$H_2O$, 3:7) comparison with authentic compound (Sigma, St. Louis, MO).

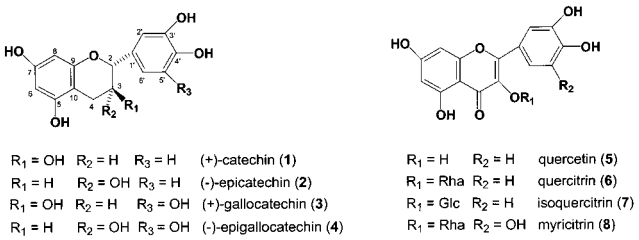


**Figure 1.** Structures of antioxidant flavonoids from *C. cainito* fruits.

**(−)-Epicatechin (2).** The separation gave 27.5 mg of white powder; yield 7.3 mg/kg fresh weight. $^1H$ and $^{13}C$ NMR are consistent with previously published data (*9*, *11*). The identification was further supported by RP18-TLC (MeOH/$H_2O$, 3:7) comparison with authentic compound (Sigma, St. Louis, MO).

**(+)-Gallocatechin (3).** The separation gave 19.8 mg of white powder; yield 5.3 mg/kg fresh weight. Negative ESIMS *m/z* 305 $[M - 1]^-$. $^1H$ and $^{13}C$ NMR are consistent with previously published data (*9*). The identification was further supported by RP18-TLC (MeOH/$H_2O$, 3:7) comparison with authentic compound (Sigma, St. Louis, MO).

**(−)-Epigallocatechin (4).** The separation gave 5.3 mg of white powder; yield 1.4 mg/kg fresh weight. Negative ESIMS *m/z* 305 $[M - 1]^-$. $^1H$ and $^{13}C$ NMR are consistent with previously published data (*12*). The identification was further supported by RP18-TLC (MeOH/$H_2O$, 3:7) comparison with authentic compound (Sigma, St. Louis, MO).

**Quercetin (5).** The separation gave 1.8 mg of yellow powder; 0.5 mg/kg fresh weight. Negative ESIMS *m/z* 301 $[M - 1]^-$. $^1H$ and $^{13}C$ NMR are consistent with previously published data (*10*, *12*). The identification was further supported by RP18-TLC (MeOH/$H_2O$, 3:7) comparison with authentic compound (Sigma, St. Louis, MO).

**Quercitrin (6).** The separation gave 7.2 mg of yellow powder; 1.9 mg/kg fresh weight. Negative ESIMS *m/z* 447 $[M - 1]^-$. $^1H$ and $^{13}C$ NMR are consistent with previously published data (*10*, *13*). The identification was further supported by RP18-TLC (MeOH/$H_2O$, 3:7) comparison with authentic compound (Sigma, St. Louis, MO).

**Isoquercitrin (7).** The separation gave a yellow powder. Negative ESIMS *m/z* 463 $[M - 1]^-$. $^1H$ and $^{13}C$ NMR are consistent with previously published data (*13*).

**Myricitrin (8).** The separation gave a yellow powder. Negative ESIMS *m/z* 463 $[M - 1]^-$. $^1H$ and $^{13}C$ NMR are consistent with previously published data (*10*).

**Gallic Acid.** The separation gave 5.8 mg of colorless needles; yield 1.6 mg/kg fresh weight. Negative ESIMS *m/z* 169 $[M - 1]^-$. $^1H$ and $^{13}C$ NMR data are in agreement with published reports (*14*). The identification was further supported by TLC comparison with authentic compound (Sigma, St. Louis, MO).

**DPPH Assay.** The DPPH assay was performed on fractions and purified isolates as previously described (*15*). In this assay, reaction mixtures containing an ethanolic solution of 400 $\mu$M DPPH (150 $\mu$L) and 2-fold serial dilutions of a fraction or purified constituent (dissolved in 50 $\mu$L dimethyl sulfoxide (DMSO)) were placed in a 96 well microtiter plate and incubated at 37 °C for 30 min. Final concentrations of test materials were typically 50, 25, and 12.5 $\mu$g/mL. After incubation, the absorbance was read at 515 nm, and mean values were obtained from duplicate readings. Antioxidant activity was determined as a percent inhibition by sample treatment by comparison with DMSO-treated controls. $IC_{50}$ values were obtained through extrapolation from linear regression analysis. $IC_{50}$ values obtained signified the concentration of sample necessary to scavenge 50% of DPPH free radicals.

**Instrumentation.** $^1H$ NMR and $^{13}C$ NMR spectra were measured on a JEOL GX-400 instrument (Akishima, Japan) operating at 400 and 100 MHz, respectively. Compounds were measured in $CD_3OD$. ESIMS in the negative mode was performed on a Finnigan LC-Q Deca instrument from Thermoquest (San Jose, CA) equipped with Excalibar software. Samples were dissolved in MeOH and introduced by direct injection. The capillary voltage was at 10 V, the spray voltage was 4.5 kV, and the tube lens was offset at 0 V. The capillary temperature was 230 °C.

**Materials.** TLC analysis was performed on silica gel 60 $F_{254}$ (EM Science, Darmstadt, Germany) plates (silica gel, 0.25 mm layer

**Table 1.** DPPH Scavenging Activity and Yields of Antioxidant Constituents of *C. cainito*

| compd | DPPH activity $IC_{50}$ ($\mu$M) | yield (mg/kg fresh weight) |
|---|---|---|
| **1** | 49.0 | 2.5 |
| **2** | 49.0 | 7.3 |
| **3** | 50.3 | 5.3 |
| **4** | 44.1 | 1.4 |
| **5** | 40.7 | 0.5 |
| **6** | 74.3 | 1.9 |
| **7** and **8** (mixture) | 56.4 | 2.4 |
| gallic acid | 42.4 | 1.6 |

thickness), with compounds visualized by spraying with vanillin in 10% (v/v) $H_2SO_4$ or 2% (w/w) $FeCl_3$. Silica gel (Merck 60 A, 230−400 mesh ASTM), Sephadex LH-20 (25−100 $\mu$m; Pharmacia Fine Chemicals, Piscataway, NJ), and RP18 silica gel (40 $\mu$m; J. T. Baker, Phillipsburg, NJ) were used for CC. RP18 $F_{254}$ plates, 1 mm layer thickness, from EM Science (Darmstadt, Germany) were also used in preparative TLC. All solvents from chromatographic isolation were of analytical grade. The DPPH was obtained from Sigma Chemical Co. (St. Louis, MO).

## RESULTS AND DISCUSSION

The fresh fruits of *C. cainito* were extracted with MeOH and then partitioned with hexane and EtOAc. The EtOAc soluble fraction displayed high antioxidant activity ($IC_{50} = 22$ $\mu$g/mL) with DPPH, a free radical compound that has been widely used to measure the radical free scavenging ability of various plant extracts and constituents (*16*, *17*). The EtOAc fraction then underwent activity-guided fractionation, initially over silica gel using VLC. Antioxidant fractions were further separated using Sephadex LH-20, RP18 CC, and preparative RP18 TLC, and nine known antioxidants were obtained.

Four flavan-3-ols, **1**−**4**, were isolated from *C. cainito*. These compounds were identified through $^1$H NMR, $^{13}$C NMR, and ESIMS. Their identification was further supported by the comparison with authentic samples on TLC. All four of these compounds are known antioxidants, and in the DPPH assay, they have similar $IC_{50}$ values, in the range of 44−50 $\mu$M (**Table 1**). Compound **2** is the antioxidant present in the highest concentration (7.3 mg/kg fresh weight) in the EtOAc soluble fraction of *C. cainito* fruits (**Table 1**). The flavan-3-ol antioxidants have been studied extensively due to their presence in tea (*18*). In a number of in vitro assays, these compounds have been shown to be potent antioxidants (*19*). For example, the tea catechins prevent the oxidation of plasma low-density lipoprotein (LDL), and because LDL oxidation is recognized as an important step in the development of cardiovascular disease, these compounds are considered to be important cardioprotective agents (*20*−*22*). Furthermore, epidemiological studies have demonstrated that the intake of tea polyphenolics is associated with a decreased risk of cardiovascular disease (*23*).

Other antioxidant flavonoids isolated from *C. cainito* include **5**−**8**. These compounds were also identified through $^1$H NMR, $^{13}$C NMR, and ESIMS. Compound **5** and its glycosides are known antioxidants found in many food plants. Compound **5** ($IC_{50} = 40.7$ $\mu$M) has a similar activity to **1** (**Table 1**). Some epidemiological studies point to the important role of **5** in the prevention of cardiovascular disease (*24*). In vivo studies indicate that **5** itself is not found in the circulatory system, but rather, conjugated metabolites of **5** participate in blood plasma's antioxidant defense (*25*). Compound **8** is found in many edible plants, and its aglycone has both antioxidant and prooxidative properties (*26*). The antioxidant gallic acid was also isolated from *C. cainito*. Gallic acid is a well-known antioxidant found in many edible plants, such as grapes (*27*).

The fruit of *C. cainito* is a rich dietary source of polyphenolic antioxidants, with **2** present in the highest concentration. Because of its good taste and its antioxidant content, star apple fruits are excellent candidates for further agricultural development.

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