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Comparative Analyses of Bioactive Mammea Coumarins from Seven Parts of *Mammea americana* by HPLC-PDA with LC-MS

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Mammea americana L. is tropical plant in the Clusiaceae family that bears edible fruit. Mammea coumarins are isoprenylated derivatives of the lactones of the 2-hydroxy-*Z*-cinnamic acids that are bioactive and have limited distribution in three Clusiaceae genera. Qualitative and quantitative analyses were performed to determine the distribution of mammea coumarins in the seed nucleus, seed coat, fruit flesh, fruit skin, leaf, stem, and root of *M. americana* using high-performance liquid chromatography−photodiode array detector (HPLC-PDA) and liquid chromatography−mass spectrometry (LC-MS). Ten major mammea coumarins, mammea E/BD (**1**), mammea E/BC (**2**), mammea E/BA (**3**), mammea E/BB (**4**), mammea B/BA hydroxycyclo F (**5**), mammea B/BD (**6**), mammea B/BC (**7**), mammea B/BA (**8**), mammea B/BB (**9**), and mammea B/BA cyclo F (**10**), were isolated and identified from the seed nucleus of *M. americana* and employed as standards. The HPLC-PDA method was validated with respect to sensitivity, linearity, recovery, accuracy, and precision. The total content (w/w %) of the 10 major mammea coumarins in *M. americana* was determined to be highest in the root (0.75%), followed by the leaf (0.64%), seed nucleus (0.48%), fruit skin (0.11%), stem (0.08%), seed coat (0.02%), and fruit flesh (<0.01%). The leaf and seed nucleus are rich and sustainable natural sources of mammea coumarins. Additionally, the described HPLC-PDA and LC-MS methods are sensitive and accurate and can be applied to the analysis of mammea coumarins in other samples.

KEYWORDS: Clusiaceae; *Mammea americana* L.; mammea coumarins; qualitative analysis; quantitative analysis; HPLC-PDA; LC-MS

INTRODUCTION

Mammea coumarins are isoprenylated derivatives of the lactones of the 2-hydroxy-*Z*-cinnamic acids from *Mammea americana* L. (Clusiaceae) and other species of *Mammea*, *Mesua*, and *Calophyllum*. About 120 mammea coumarins have been isolated and identified from plants (*1*−*20*). Mammea coumarins express a wide range of bioactivities, such as insecticidal (*1*, *21*), antioxidant (*2*), anticancer (*2*, *13*, *16*, *17*, *22*), anti-HIV (*18*−*20*, *23*, *24*), antifungal (*14*), antibacterial (*15*), antimicrobial (*12*), and antibiotic activities (*25*).

M. americana is a tree native to the West Indies and northern South America that bears round fruits 10−20 cm in diameter. It has a light brown skin and orange flesh with one to four large ovoid seeds per fruit. The ripe fruit is eaten fresh, with wine, sugar, and cream, or made into sauces, pies, tarts, and jams. Preserves made from the pulp are said to taste remarkably like apricot preserves (*26*). Mature green fruits are high in pectin and make an excellent jelly when combined with high-acid fruits that are low in pectin, such as pineapple. From the fragrant white flowers a liqueur is distilled, which is known as eau de créoloe or crême de créole. In Brazil, fermented toddy is made from the sap of the tree (*26*, *27*). The leaves of *M. americana* are also edible and used to prepare a digestive drink (*28*).

Extracts and pure compounds obtained from *M. americana* have insecticidal and larvicidal activities (*1*, *21*, *29*, *30*). Traditionally in the West Indies the ground seeds are used as an insect powder (*21*). In Mexico and Jamaica, the thick, yellow gum from the bark is melted with fat and applied to the feet to combat chiggers and used to rid animals of fleas and ticks. A greenish yellow, gummy resin from the skin of immature fruits and an infusion of half-ripe fruit are similarly employed. In El Salvador, a paste made of the ground seeds is used against poultry lice, mites, and head lice. In Puerto Rico, the leaves of

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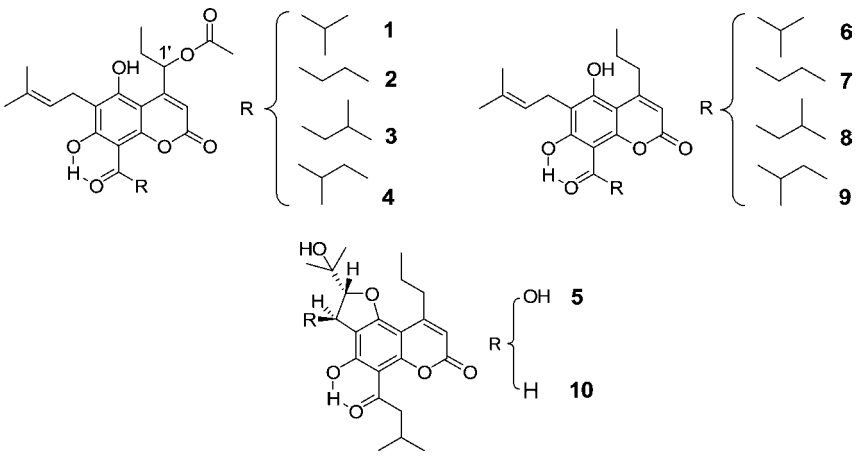

Figure 1. Chemical structures of 10 mammea coumarin standards from *M. americana*.

M. americana are wrapped around young tomato plants to protect them from mole crickets and cutworms (*26*). Whole plant extracts have demonstrated insecticidal activity on *Aedes aegyptii* (*31*), and extracts of the seeds have larvicidal activity against *Laphygma* sp. and *Plutella* sp. (*32*). The ethanolic leaf extract has strong anti-mycobacterium tuberculosis properties (*33*). Mammein and other coumarins isolated from *M. americana* have antitumor activity (*3*).

Thirty-nine mammea coumarins have previously been isolated and identified from *M. americana*, including three new coumarins we identified (*1*−*9*, *11*). Some of these compounds were elucidated from isomeric mixtures in the 1960s and 1970s, due to the difficulty of separating these structurally similar coumarins. To the best of our knowledge, there is no other study on the qualitative and quantitative analyses of mammea coumarin mixtures by high-performance liquid chromatography−photodiode array detection (HPLC-PDA) and/or liquid chromatography−mass spectrometry (LC-MS). In this study, preparative HPLC was used to isolate 10 compounds, mammea E/BD (**1**), mammea E/BC (**2**), mammea E/BA (**3**), mammea E/BB (**4**), mammea B/BA hydroxycyclo F (**5**), mammea B/BD (**6**), mammea B/BC (**7**), mammea B/BA (**8**), mammea B/BB (**9**), and mammea B/BA cyclo F (**10**), from the seed nucleus of *M. americana* (*2*). Qualitative and quantitative analyses were performed to determine the distribution of mammea coumarins in the seed nucleus, seed coat, fruit flesh, fruit skin, leaf, stem, and root of *M. americana* using HPLC-PDA and LC-MS. Here we report the results of qualitative and quantitative analyses of these 10 mammea coumarins from seven parts of *M. americana*.

MATERIALS AND METHODS

Plant Material. Fruits, leaves, stems, and roots of *M. americana* were collected from the Fruit and Spice Park (Homestead, FL). Fresh fruits, leaves, stems, and roots were shipped to New York City by overnight courier and stored at −20 °C until extracted. A voucher specimen of this plant material was prepared, identified, and deposited at the herbarium of The New York Botanical Garden (Bronx, NY).

Instrumentation. Electrospray ionization mass spectrometry (ES-IMS) was performed with a ThermoFinnigan LCQ instrument (San Jose, CA) equipped with Xcalibur software. HPLC analyses were carried out on a Waters 2695 Separations Module (Milford, MA) equipped with a model 996 photodiode array detector and Empower software using a 250 × 4.6 mm i.d., 5 μm, Aqua C_{18} column (Phenomenex, Torrance, CA). Preparative HPLC was carried out using a Waters 600 controller with a model 486 tunable absorbance detector and Empower software with a 250 × 21.1 mm i.d., 10 μm, Nucleosil C_{18} column (Phenomenex) and an isocratic solvent system of MeCN/H_2O (75:25), a flow rate of 5 mL/min, the column at room temperature, and a 50 min run time. TLC analyses were performed on silica gel 60 F_{254} (EM Science, Darmstadt, Germany) and RP-18 F_{254} plates (EM Science), with compounds visualized by spraying with a vanillin solution (1.0 g of vanillin in 10 mL of concentrated H_2SO_4 and 90 mL of EtOH) and heating at ≈50 °C. Silica gel (230−400 mesh) (EM Science), silica gel 60 (particle size = 0.015−0.040 mm) (EM Science), Sephadex LH-20 (25−100 μm) (Pharmacia Fine Chemicals, Piscataway, NJ), and C_{18} reversed-phase silica gel (40 μm) (J. T. Baker, Phillipsburg, NJ) were used for column chromatography.

Extraction and Isolation. The fresh seed nuclei (487.3 g) were ground and exhaustively extracted with MeOH three times at room temperature. The combined MeOH extract solution was concentrated to dryness in vacuo, and the resulting extract (35.7 g) was suspended in H_2O and partitioned with EtOAc. The EtOAc partition fractions (6.2 g) of seed nuclei were obtained after removal of EtOAc in vacuo. The fresh seed coat (177.0 g), fruit flesh (451.9 g), fruit skin (307.6 g), leaf (200.6 g), stem (183.3 g), and root (70.3 g) of *M. americana* were processed using the same fashion as fresh seed nucleus. The MeOH extracts of seed coat (9.3 g), fruit flesh (38.0 g), fruit skin (22.4 g), leaf (19.9 g), stem (15.8 g), and root (6.6 g) and the EtOAc partition fractions of seed coat (0.9 g), fruit flesh (1.1 g), fruit skin (4.3 g), leaf (8.8 g), stem (3.2 g), and root (3.3 g) were obtained, respectively.

Ten mammea coumarins, mammea E/BD (**1**), mammea E/BC (**2**), mammea E/BA (**3**), mammea E/BB (**4**), mammea B/BA hydroxycyclo F (**5**), mammea B/BD (**6**), mammea B/BC (**7**), mammea B/BA (**8**), mammea B/BB (**9**), and mammea B/BA cyclo F (**10**), were isolated from the fresh seed nuclei (4.0 kg) of *M. americana* as described previously (*2*) and used as standards in this study. Their structures are shown in **Figure 1**.

Preparation of Samples for HPLC and LC-MS Analyses. The EtOAc fractions of the seven different parts of *M. americana* were redissolved in HPLC grade MeOH to make 5 mg/mL sample solutions. All samples were filtered with a 0.45 μm nylon microfilter (Phenomenex) before HPLC and LC-MS analyses.

Preparation of Standards for HPLC Analyses. Stock solutions (1 mg/mL) for the 10 standards were prepared by dissolving individual standards in HPLC grade MeOH. The stock solutions were diluted with HPLC grade MeOH into seven serial solutions, respectively. The concentrations of individual standards in the seven 2-fold serial solutions were 0.008, 0.016, 0.031, 0.063, 0.125, 0.25, and 0.50 mg/mL.

HPLC Solvent Gradient Profile. HPLC conditions included the use of a 250 × 4.6 mm i.d., 5 μm, Aqua C_{18} column (Phenomenex) at 25 °C with a flow rate of 1.0 mL/min. The solvent system consisted of 10 mM ammonium acetate in deionized water (solvent A) and acetonitrile (solvent B). The gradient profile was as follows: sample analysis (0−10 min, 65−55% A; 10−20 min, 55−55% A; 20−30 min, 55−45% A; 30−35 min, 45−45% A; 35−40 min, 45−35% A; 40−60 min, 35−35% A; 60−70 min, 35−65% A) and column wash and equilibration (70−80 min, 65−65% A). An injection volume of 10 μL was used. The HPLC chromatograms were extracted at 332 nm.

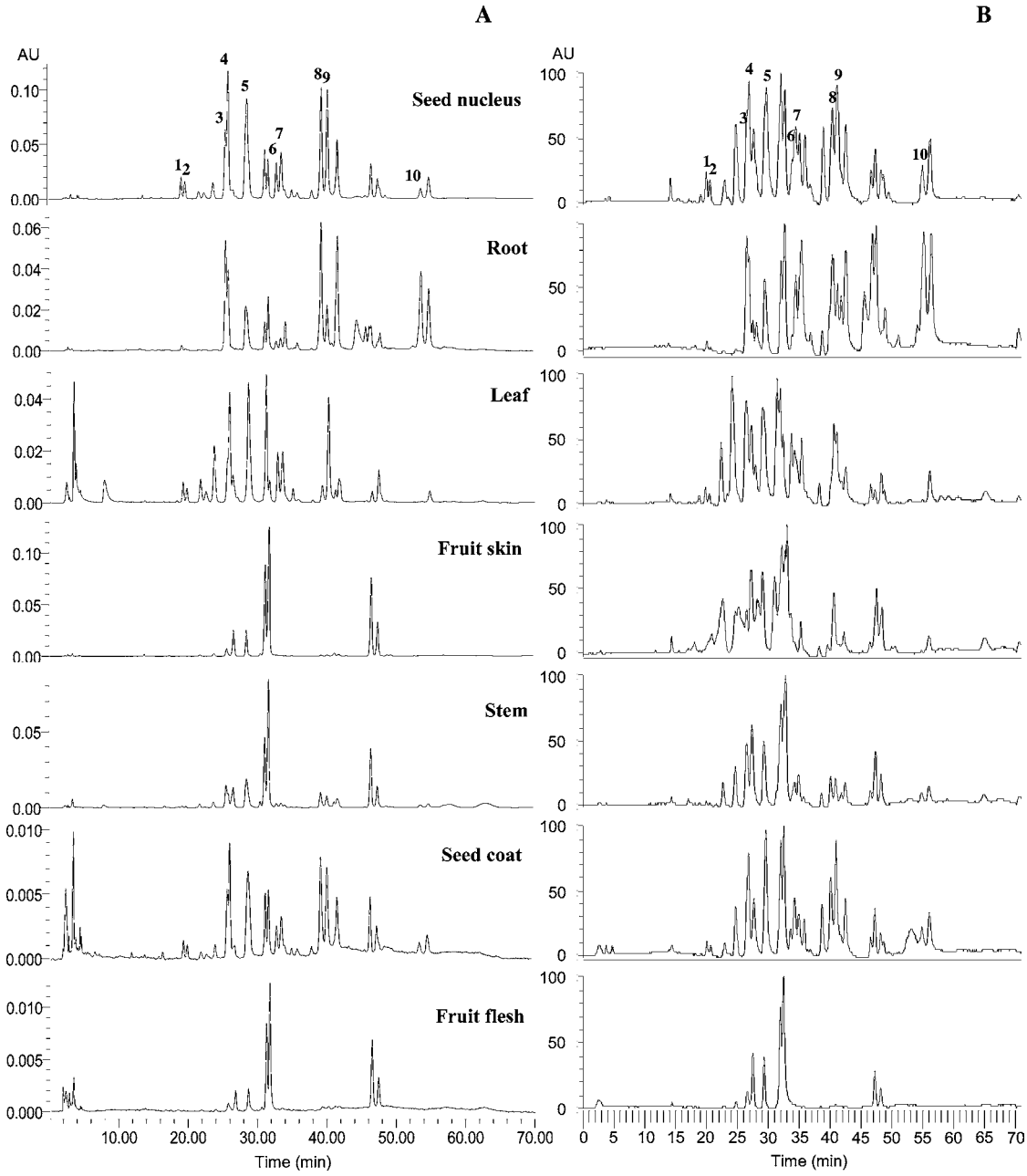

Figure 2. HPLC-PDA (332 nm) chromatograms (**A**) and LC-ESI-MS total ion current (TIC) chromatograms (**B**) of the EtOAc partition fractions of seven parts of *M. americana*.

LC-ESI-MS Analysis. For qualitative analysis, samples and standards (10 μL) were separated using the same HPLC method described previously. Column effluent directed to the ESI probe on the mass spectrometer was monitored in full scan mode for negative ions of samples and standards. The capillary voltage was 10 V, the spray voltage was 4.5 kV, and the tube lens offset was 0 V. The capillary temperature was 230 °C. Nitrogen was used as both sheath and auxiliary gas, and their flow rates were 80 and 30 (arbitrary units), respectively.

Calibration Curves, Recoveries, and Detection limits. Calibration curves were generated for each of the 10 standards after runs of the seven 2-fold serial solutions.

The recoveries for the analytical method were evaluated by adding 0.1 mg of mammea B/BA (**8**) and 0.1 mg of mammea B/BA cyclo F (**10**) to each of the ground plant parts (1.0 g) of *M. americana* prior to extraction. The recoveries were determined by subtracting the values obtained for the control (unspiked) matrix preparation from the sample added with standards, divided by the amount of standards, and expressed as percentage. In these spike−recovery experiments, each of the ground plant parts (1.0 g) of *M. americana* spiked with 0.1 mg of mammea B/BA (**8**) and 0.1 mg of mammea B/BA cyclo F (**10**) was extracted with MeOH three times (3 × 10 mL), at room temperature for 1 h each. The MeOH extract was concentrated in vacuo to dryness and dissolved with HPLC grade MeOH to prepare 1 mg/mL solution. This solution was quantified for mammea B/BA (**8**) and mammea B/BA cyclo F (**10**) using HPLC-PDA triplicate injections, and a percent recovery was calculated.

The limit of detection (LOD) was calculated as ratios of signal-to-noise with nominal values of 3:1. LOD experiments were evaluated by performing triplicate injections of standard solutions at the LOD concentration. Mammea B/BA (**8**) and mammea B/BA cyclo F (**10**) were selected to perform recovery and LOD studies; compound **8** has structural and UV absorbance similarities to compounds **1**−**4** and **6**−**9**, and compound **10** has structural and UV absorbance similarities to compound **5**.

RESULTS AND DISCUSSION

Due to the significant bioactivity of mammea coumarins and their potential use in agriculture, food, and medicine, we developed an HPLC-PDA method to quantify these compounds

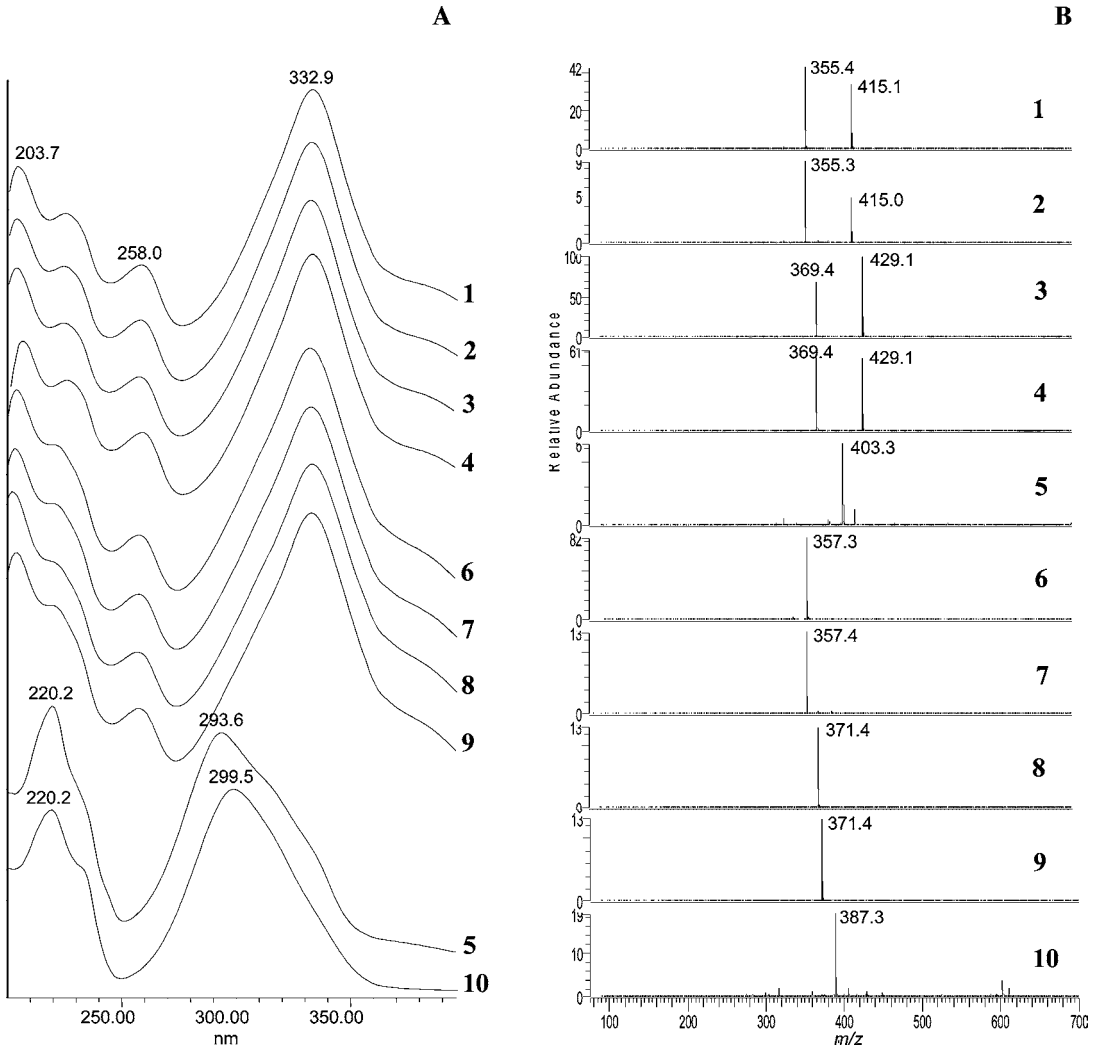

Figure 3. UV spectra (**A**) detected by HPLC-PDA and MS spectra (**B**) detected by negative ESI-MS of 10 mammea coumarin standards (**1**–**10**).

in seven different parts of the *M. americana* plant. To optimize an HPLC method, many different solvent systems were tested, including MeOH/water, MeCN/water, acetone/water, MeOH or MeCN mixtures with aqueous formic or acetic acid, and MeOH or MeCN with aqueous ammonium acetate. Both isocratic and gradient programs were tested in combination with three different columns: Aqua C_{18}; Nucleosil C_{18}; and Luna C_{18}. The 250 × 4.6 mm i.d., 5 μm, Aqua C_{18} column and HPLC solvent gradient program described previously provided the best separation among the mammea coumarins. The resulting HPLC and LC-MS chromatograms show good separation among most of the constituents, although the baseline separation could not be achieved for all of the mammea coumarins in the *M. americana* samples (**Figure 2**).

Mammea coumarins are well suited for HPLC-PDA analysis, because they have strong UV absorbance and characteristic UV spectra as shown in **Figure 3**. The UV spectra of **1**−**4** are identical with those of **6**−**9**, whereas **5** and **10** exhibited similar UV spectra, due to the similarities of their structures. The UV spectra of **5** and **10** are different from those of **1**−**4** and **6**−**9** due to the addition of a furan ring in **5** and **10**. However, it is easy to distinguish the mammea coumarins from the other classes of compounds (e.g., flavonoids and xanthones) in *M. americana* by their UV spectra.

Compounds **1**−**10** in each sample were identified by comparing their retention time (t_R), UV, and MS spectra with those of individual standards (**Table 1** and **Figure 3**). Peak areas for

Table 1. Retention Times and Mass Fragments of **1**–**10**

mammea compound	$t_R \pm$ SD (min)	$[M - H]^-$	$[M - AcOH - H]^-$ (*1*, *2*)
E/BD (**1**)	19.02 ± 0.05[a]	415.1	355.4
E/BC (**2**)	19.46 ± 0.04	415.0	355.3
E/BA (**3**)	25.27 ± 0.03	429.1	369.4
E/BB (**4**)	25.65 ± 0.03	429.1	369.4
B/BA hydroxycyclo F (**5**)	28.32 ± 0.01	403.3	
B/BD (**6**)	32.64 ± 0.03	357.3	
B/BC (**7**)	33.28 ± 0.01	357.4	
B/BA (**8**)	39.07 ± 0.01	371.4	
B/BB (**9**)	40.01 ± 0.02	371.4	
B/BA cyclo F (**10**)	53.19 ±0 .05	387.3	

[a] Mean ± SD (n = 4).

1−**10** and individual standards were integrated from the HPLC-PDA chromatograms and used for quantification. Individual calibration curves for each standard measured in the HPLC-PDA analyses are linear, with a zero intercept and correlation coefficient (γ^2) values >0.99 in the range (0.008−0.5 mg/mL) of calibration of the standards tested. In this HPLC-PDA method, the LODs were determined as 0.8 ng for mammea B/BA (**8**) and 1.5 ng for mammea B/BA cyclo F (**10**). Spike−recovery experiments were performed on each of the seven parts of *M. americana* used in this study. For mammea B/BA, the recovery for all matrices was >93%, with a range from 93.7 ± 0.3% (seed nucleus) to 99.4 ± 0.9% (fruit flesh). For mammea

Table 2. Quantification of Mammea Coumarins (**1**–**10**) in Seven Parts of *M. americana* Determined by HPLC-PDA

mammea compound	root (w/w%)[a]	leaf (w/w%)	seed nucleus (w/w%)	fruit skin (w/w%)	stem (w/w%)	seed coat (w/w%)	fruit flesh (w/w%)
E/BD (**1**)	$0.01 \pm 0.5 \times 10^{-3}$ [b]	$0.03 \pm 0.44 \times 10^{-3}$	$0.02 \pm 0.13 \times 10^{-3}$	ND[c]	Tr[d]	Tr	ND
E/BC (**2**)	Tr	$0.02 \pm 0.44 \times 10^{-3}$	$0.02 \pm 0.38 \times 10^{-3}$	ND	ND	Tr	ND
E/BA (**3**)	$0.23 \pm 4.7 \times 10^{-3}$	$0.24 \pm 1.13 \times 10^{-3}$	$0.07 \pm 2.87 \times 10^{-3}$	$0.01 \pm 0.16 \times 10^{-3}$	$0.02 \pm 0.40 \times 10^{-3}$	Tr	Tr
E/BB (**4**)	$0.16 \pm 1.3 \times 10^{-3}$	$0.06 \pm 1.01 \times 10^{-3}$	$0.12 \pm 0.43 \times 10^{-3}$	$0.03 \pm 0.58 \times 10^{-3}$	$0.03 \pm 0.35 \times 10^{-3}$	Tr	Tr
B/BA hydroxy-cyclo F (**5**)	$0.05 \pm 0.5 \times 10^{-3}$	$0.07 \pm 1.10 \times 10^{-3}$	$0.06 \pm 0.38 \times 10^{-3}$	$0.07 \pm 5.00 \times 10^{-3}$	$0.01 \pm 0.33 \times 10^{-3}$	Tr	Tr
B/BD (**6**)	$0.01 \pm 0.9 \times 10^{-3}$	$0.05 \pm 1.10 \times 10^{-3}$	$0.02 \pm 0.46 \times 10^{-3}$	ND	Tr	Tr	ND
B/BC (**7**)	$0.03 \pm 2.1 \times 10^{-3}$	$0.05 \pm 1.32 \times 10^{-3}$	$0.03 \pm 0.32 \times 10^{-3}$	ND	Tr	Tr	ND
B/BA (**8**)	$0.15 \pm 6.6 \times 10^{-3}$	$0.02 \pm 0.50 \times 10^{-3}$	$0.07 \pm 0.93 \times 10^{-3}$	Tr	$0.01 \pm 0.35 \times 10^{-3}$	Tr	Tr
B/BB (**9**)	$0.06 \pm 0.7 \times 10^{-3}$	$0.10 \pm 1.24 \times 10^{-3}$	$0.07 \pm 0.67 \times 10^{-3}$	Tr	$0.01 \pm 0.09 \times 10^{-3}$	Tr	Tr
B/BA cyclo F (**10**)	$0.05 \pm 3.1 \times 10^{-3}$	Tr	Tr	ND	ND	ND	ND
total amount (w/w%)	0.75 ± 0.012	0.64 ± 0.006	0.48 ± 0.004	0.11 ± 0.006	0.08 ± 0.001	0.02 ± 0.0003	<0.01

[a] Amount of compound in fresh plant material. [b] Mean ± SD ($n = 4$). [c] ND, not detected. [d] Tr, amount of compound in fresh plant material is <0.01%.

B/BA cyclo F, the recovery for all matrices was >90%, with a range from 90.3 ± 0.3% (leaf) to 95.8 ± 0.5% (fruit flesh).

The amount of each of the 10 mammea coumarins was measured by HPLC-PDA in the seven parts of *M. americana* and is reported as the total of these 10 compounds (w/w %) (**Table 2**). The root of *M. americana* has the highest amount of the 10 coumarins (0.75%), followed by the leaf (0.64%), seed nucleus (0.48%), fruit skin (0.11%), stem (0.08%), seed coat (0.02%), and fruit flesh (<0.01%). The leaf and seed nucleus have higher content of mammea coumarins. In addition, the seed is a major part (w/w %) of the fruit of *M. americana*. The yield of the seed nucleus from the fruit was up to 22.7% (w/w %) in our study.

Comparing the HPLC-PDA chromatograms with those obtained from LC-ESI-MS of seven samples, we found that the HPLC-PDA method (extracted at 332 nm) showed higher resolution for **1**−**10** than did the LC-ESI-MS full scan; therefore, we used the HPLC-PDA for quantitative analysis. In addition, other LC-MS techniques for enhancing the sensitivity or specificity, such as selected ion monitoring (SIM) and selected reaction monitoring (SRM), cannot be applied in this case, because each sample contains many isomers that may overlap and produce false positive results. Despite its limitations, LC-ESI-MS full scan mode provided important information (e.g., molecular and fragment ions) to complement the HPLC-PDA analysis.

Mammea coumarins are well-known natural products especially in the defense against insects and fungi in agriculture (*21*). The insecticidal activity of 26 allied mammea coumarins was shown to result from the inhibition of oxidative phosphorylation, but none of the 26 displayed topical insecticidal activity (*1*, *21*). Two additional coumarins isolated from *M. americana*, mammea E/BA (**3**) and E/BB (**4**), are topically insecticidal as well as being inhibitors of oxidative phosphorylation (*1*). These two compounds have substantially stronger insecticidal activity toward mustard beetles and houseflies than the other 26 mammea coumarins (*1*). Side-chain acetoxylation at the 1′-position was determined to be important for the expression of topical insecticidal activity in these mammea coumarins (*1*, *21*). Our previous investigation of *M. americana* resulted in the isolation and identification of two new mammea coumarins, mammea E/BC (**2**) and E/BD (**1**), with the side-chain acetoxyl at the 1′-position (*2*), and on the basis of structure−activity relationships, these two new compounds may be topically insecticidal as well. In addition, mammea E/BA (**3**) and E/BB (**4**) were previously reported only from the seed of *M. americana* (*1*). We detected both of these compounds in all seven parts of *M. americana*, the root having the highest concentration (0.39%, w/w), followed by the leaf (0.30%), seed nucleus (0.19%), stem (0.05%), fruit skin (0.04%), seed coat (<0.01%), and fruit flesh (<0.01%) (**Table 2**). Our studies show that, in addition to the seed, the root and leaf are also rich sources of insecticidal mammea coumarins. These results explain why the whole plant extracts of *M. americana* have insecticidal activity and also explain why leaves are used in many cultures as insecticides.

Mammea coumarins have also been widely studied for their pharmacological activities (*1*, *2*, *12*−*25*). As part of our continuing research on polyphenolic anticancer agents and antioxidants from edible plants (*2*, *34*−*37*), we found that methanolic extracts of *M. americana* seeds have high antioxidant and cytotoxic activities. Bioactivity-guided fractionation of *M. americana* seeds resulted in the identification of (−) catechin, (−) epicatechin, and 15 mammea coumarins (*2*). These 15 mammea coumarins exhibited significant cytotoxic activities in the SW-480, HT-29, and HCT-116 human colon cancer cell lines at concentrations comparable to those of 5-fluorouracil, a drug frequently used for human colon cancer treatment. Ten of these mammea coumarins displayed high antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (*2*). The mammea coumarins with the side-chain acetoxyl at the 1′-position, mammea E/BD (**1**), mammea E/BC (**2**), mammea E/BA (**3**), and mammea E/BB (**4**), were also proven to have the highest antioxidant and cytotoxic activities among the 15 mammea coumarins. In this study, we found that all seven parts of *M. americana* contain antioxidant and anticancer mammea coumarins (**Table 2**). In *M. americana*, the quantity of the most potent antioxidant and anticancer mammea coumarins (**1**−**4**) was the highest in root (0.40%), followed by the leaf (0.35%), seed nucleus (0.23%), stem (0.05%), fruit skin (0.04%), seed coat (<0.01%), and fruit flesh (<0.01%).

In conclusion, the described method provides a sensitive and reproducible technique for the quantification of mammea coumarins in different parts of *M. americana*. The leaf and seed nucleus of *M. americana* are rich and sustainable natural sources of mammea coumarins for development as insecticidal, antioxidant, and cancer chemopreventive agents. Even though the content of mammea coumarins is relatively low in the fruit flesh, because it is edible and flavorful, it may be important as a dietary source of antioxidants and cancer chemopreventive compounds. Further studies on the safety and efficacy mammea coumarins are needed in order for them to be used in agriculture, cosmetics, and pharmaceutics. We are currently studying the mechanism of cytotoxicity in colon cancer cells and other medical values of these mammea coumarins. There is great promise for the

use of *M. americana* to create new health and agricultural products from sustainable sources.

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