

SUPPLEMENTARY MATERIAL

GC-MS analysis of the bioactive phytoconstituents of various organic crude extracts from the seed kernels of *Manilkara bidentata* (balata) collected in Trinidad, W.I.

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Abstract

The present study reports the first phytochemical investigation of the seed kernels of *Manilkara bidentata* (Balata) harvested in Trinidad, W.I. Gas chromatography-mass spectrometry (GC-MS) analysis of the n-hexane, chloroform, ethyl acetate, ethanol and methanol extracts showed a total of 39 components. 2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene (74.93%), 9-octadecenoic acid, (Z)- 2,3-dihydroxypropyl ester (79.98%), (Z)-ethyl oleate (92.75%), Z,E-2-methyl-3,13-octadecadien-1-ol (80.51%) and 5-(hydroxymethyl)-2-furancarboxyaldehyde (50.32%) were the major constituents identified in the n-hexane, chloroform, ethyl acetate, ethanol and methanol extracts, respectively. The extracts showed the presence of several bioactive components and provides reference data for further research of its active constituents.

Keywords: *Manilkara bidentata*; Sapotaceae; balata; seed kernel extracts; GC-MS; bioactive phytochemicals; Trinidad

Experimental

Plant material: The fruits of *Manilkara bidentata* were purchased in April 2016 from a retail market in East Trinidad, when they were ripe and edible. Identification and botanical authentication was performed by the National Herbarium of Trinidad and Tobago where a voucher specimen (TRIN 40644) was deposited.

Sample preparation and extraction: The seeds were removed from the fruits, washed thoroughly with distilled water and air-dried at room temperature for 2 days. The black, hard outer shells of the seeds were cracked manually and removed to obtain the seed kernels (50% of the whole seed) which were dried in an oven at 40 °C overnight. Dried seed kernels were pulverized to a fine powder using a domestic grinder (Hamilton Beach 80333). 30 g quantities of the dried powdered material were weighed into five separate glass bottles. Individual 300 mL volumes of n-Hexane, Chloroform, Ethyl acetate, Ethanol and Methanol were added to the ground material and extracted by sonication (Bransonic 2510 Ultrasonic Bath) for 2 hours at room temperature. The extracts were subsequently filtered through filter paper (Whatman No. 1) and evaporated at 50 °C under reduced pressure using a rotary evaporator (Buchi RII). The percentage yields for the five extracts were 4.34%, 8.63%, 10.23%, 17.77% and 22.37% for the n-Hexane (pale yellow colour), Chloroform (light brown colour), Ethyl acetate (yellow colour), Ethanol (yellow colour) and Methanol (orange colour), respectively. The

percentage yields were calculated as grams of extract divided by grams of original powder on a dry weight basis (w/w). The thick colloidal extracts obtained were stored in sterile, air tight, dark bottles and kept at 4 °C until GC-MS analyses were performed.

GC-MS analysis: Gas Chromatography-Mass Spectrometry analysis was performed by a Perkin Elmer Clarus 500 GC coupled to a Perkin Elmer Clarus 500 MS installed with a split/splitless injector and Elite-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.5 µm film thickness). The carrier gas used was Helium (99.999%) at a flow rate of 0.5 mL/min. The sample was introduced via splitless injection and the injection port was maintained at 250 °C. The oven temperature programming commenced at 50 °C and increased at a rate of 10 °C/min until 300 °C and held constant at 300 °C for 5 min. The interface temperature was kept at 220 °C. The MS scan involved the ionization technique of electron impact (EI) with a scanning range from 50 to 400 amu at 0.1 second intervals. The total GC run time was 30 min. An aliquot of C7-C40 *n*-alkanes (Sigma Aldrich) was analysed separately under the same conditions as the five extracts to obtain the retention time of each *n*-alkane necessary for the Kovats indices calculations.

Identification of Components

The eluted chemical components were first identified via GC-MS by comparing their obtained mass spectra with those listed in the authenticated National Institute of Standards and Technology (NIST) and WILEY libraries supplied with the instrument. Further identification was made by comparison of their Kovats retention indices (RI) with those reported in the literature. The Kovats retention indices were determined in relation to a homologous series of *n*-alkanes (C7-C40) under the same chromatographic conditions according to Van den Dool's method (Van den Dool and Kratz 1963).

TLC analysis and identification of triterpenoids using Liebermann-Burchard Test

All extracts were analysed using TLC and were checked for the presence of triterpenes using the Liebermann-Burchard spray reagent. After development and drying, the TLC plate was sprayed with a freshly prepared solution of Liebermann-Burchard reagent (5mL acetic anhydride + 5 mL of conc. sulphuric acid in 50 mL of absolute ethanol) and heated to 100 °C for 10 minutes. A pink or red colouration indicates the presence of triterpenoids.

Table and Figure captions

Table S1. Chemical compositions and relative amounts of the five extracts of the seed kernels of *Manilkara bidentata*.

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No.	Compounds ^a	RI ^b	Composition/%				
			H ^c	C ^c	EA ^c	ET ^c	M ^c
1	2,5-dimethyl furan	712	-	-	-	-	0.43
2	2-Furanmethanol	880	-	-	-	-	0.55
3	5-methyl-2-furancarboxaldehyde	970	-	-	-	-	3.14
4	3,9-dimethylundecane	1106	0.18	-	-	-	-
5	α -D-Glucopyranosiduronic acid, methyl, methyl ester	1130	-	-	0.65	-	-
6	2(5H)-furanone-3-hydroxy-4,5-dimethyl	1135	-	-	-	-	0.88
7	2-Propyl-tetrahydropyran-3-ol	1152	-	-	-	-	7.30
8	Benzene, 1,2,3-trimethoxy-5-methyl	1232	-	-	-	-	0.15
9	5-Acetoxymethyl-2-furaldehyde	1247	-	-	-	-	0.91
10	2,3-Pentadienoic acid-, ethyl ester	1253	-	-	0.48	-	-
11	3-methyl-2-Furoic acid	1261	-	-	-	-	7.21
12	2-methyltridecane	1270	tr ^d	-	-	-	-
13	2-Furancarboxyaldehyde, 5-(hydroxymethyl)-	1286	-	-	-	-	50.32
14	(-)-Mellein	1586	0.25	-	-	-	-
15	3-Ethoxy-4-Methoxy Phenol	1715	-	-	-	-	0.22
16	Pentadecanoic acid, 14-methyl-, methyl ester	1884	0.40	-	-	-	-
17	1-Isobutyl-7,7-Dimethyl-Octahydro-Isobenzofuran-3A-ol	1896	-	-	-	-	4.83
18	Hexadecanoic acid, ethyl ester	1981	5.32	-	-	0.22	-
19	Hexadecanoic acid	2053	2.25	-	-	3.93	-
20	Hexadecanoic acid, methyl ester	2059	-	tr ^d	0.51	-	0.22
21	Hexadecanoic acid, butyl ester	2112	0.95	-	-	-	-
22	9,12-Octadecadienoic acid, ethyl ester	2126	2.72	-	-	-	-
23	9,10-Anthracenedione-1,8-dihydroxy-3-methoxy-6-methyl	2133	-	0.58	2.16	-	0.18
24	(<i>E</i>)-9-Octadecenoic acid, ethyl ester	2138	7.21	-	-	-	-

25	Isopropyl palmitate	2146	-	0.63	1.06	-	0.33
26	Octadecanoic acid	2157	0.10	-	-	-	-
27	17-methoxyoctadecanoic acid methyl ester	2163	-	-	2.39	-	-
28	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	2178	0.33	-	-	-	-
29	(Z)-9-Octadecenoic acid, butyl ester	2182	1.53	-	-	-	0.50
30	(Z)-Ethyl Oleate	2189	-	18.73	92.75	3.04	6.57
31	(9Z,12Z)-9,12-Octadecadienoic acid	2194	-	-	-	9.16	-
32	2H-Pyran-2-one, tetrahydro-6-tridecyl-	2201	0.22	-	-	-	-
33	Z,E-2-methyl-3,13-octadecadien-1-ol	2334	-	-	-	80.51	-
34	1,2-Benzenedicarboxylic acid, diisooctyl ester	2549	3.06	-	-	0.36	-
35	Heneicosane, 11-(1-ethylpropyl)-	2605	0.21	-	-	-	-
36	9-Octadecenoic acid, (Z)-2,3-dihydroxypropyl ester	2692	-	79.98	-	-	16.26
37	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	2698	74.93	-	-	-	-
38	1-Heptacosene	2700	0.26	-	-	-	-
39	Squalene	2788	-	-	-	2.77	-
<i>Fatty acids</i>		2.35				13.09	
<i>Fatty acid esters</i>		18.46	99.42	97.19	3.26	23.89	
<i>Furan derivatives</i>						68.26	
<i>Hydrocarbon derivatives</i>		75.66				83.28	
<i>Others</i>		3.53	0.58	2.81	0.36	7.85	
<i>Identified compounds</i>		17	5	7	7	17	

^a Compounds are listed in the order of elution on the Elite-5MS fused-silica capillary column

^b RI indicates the retention indices which were calculated relative to C7-C40 n-alkanes on an Elite-5MS fused-silica capillary column

^c H – n-Hexane extract; C – Chloroform extract; EA – Ethyl acetate extract; ET – Ethanol extract; M – Methanol extract

^d tr – trace (< 0.1%).

- means not detected

References:

Van den Dool H and Kratz PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*. 11:463-471