

Antioxidant, anti-diabetic and other health-promoting properties of Jamaican *Rubus* berry species

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Presentation Overview

- Research goal and objectives
- Local (Jamaican) context
- Jamaican *Rubus* berry species
- Research methods and results
- Summary of research findings

Research Goal

Conduct research on uncommon Jamaican plant species aimed at investigating their health-beneficial properties in order to promote productive utilization of the island's plant resources.

Research Objectives

- To determine the health-beneficial properties of uncommon Jamaican fruit species
  - Antioxidant, anti-inflammatory, anticancer, antidiabetic, antihypertensive

- To isolate and characterize compounds from extracts demonstrating biological activity (bioactivity-guided approach).

- To investigate the biological activity of the pure isolates→→ identify compounds responsible for biological activity.

- To disseminate the findings via seminars, conferences and scientific journal articles →→ public awareness.

Jamaican Flora

- **3000 species of flowering plants grow on the island**
- **27% endemic**
- **Many species are generally unknown and underutilized**

Berry Imports in Ja

In 2012 Jamaica imported over £600,000 worth of fresh/frozen berry fruits (STATIN)

Jamaican Rubus Berries



R. rosifolius



R. racemosus



R. ellipticus



R. jamaicensis

Rubus

- **Family: Rosaceae**
- **12 subgenera**
- **Over 250 species**

Rubus Fruits

Raspberries



R. idaeus

Blackberries



R. fruticocus

Dewberries



R. saxatilis

Pictures courtesy of superstock.com and wikipedia.org

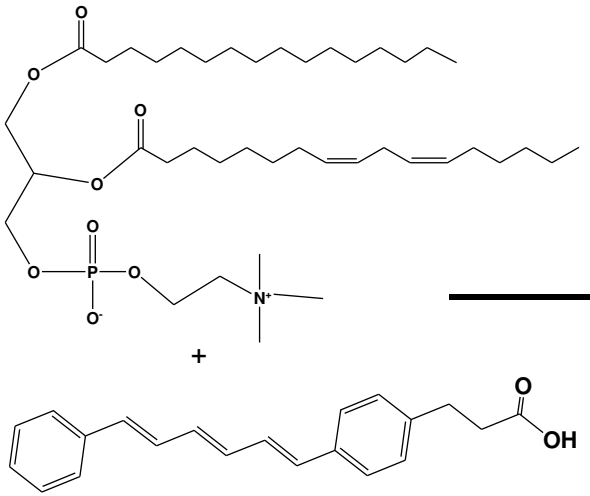
Rubus Diversity



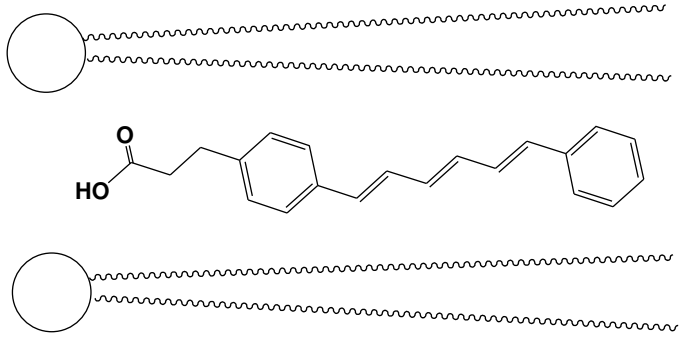
Picture courtesy of superstock.com

Methodology and Results

Antioxidant Activity Testing via Lipid Peroxidation Inhibitory Assay (LPO)



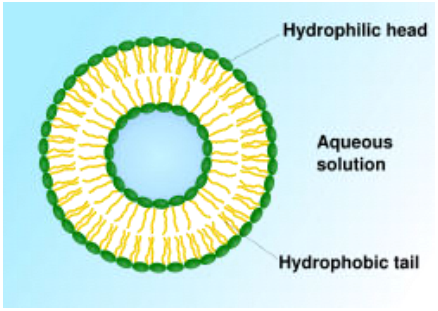
3-[p-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (DPH-PA)



large unilamellar vesicles (LUVs) with incorporated fluorescent DPH-PA probe

Addition of Fe (II)

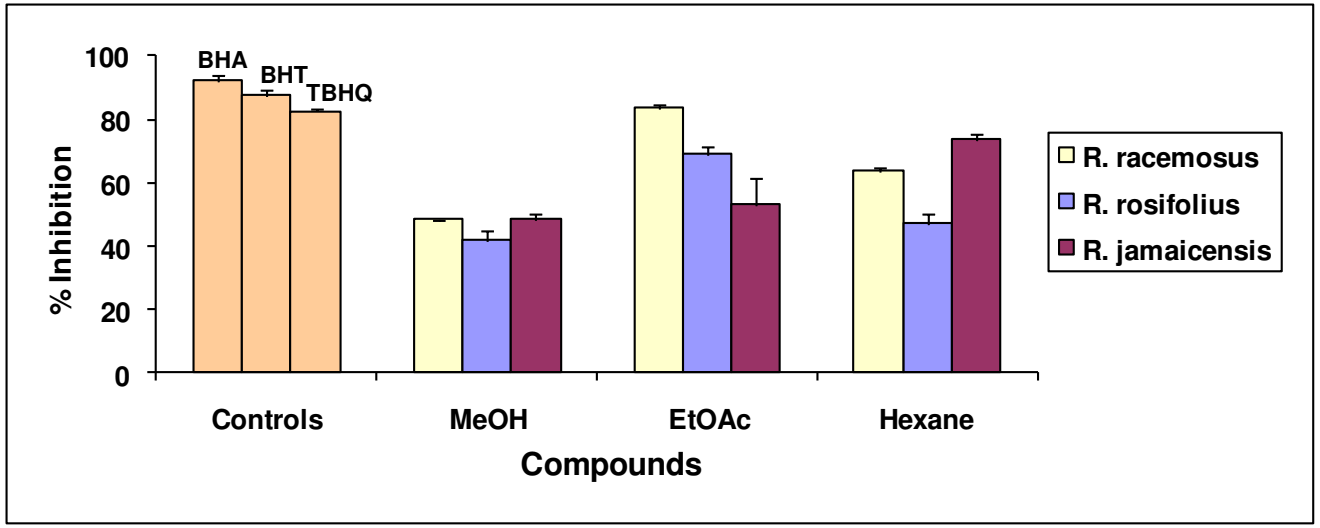
Loss of fluorescence

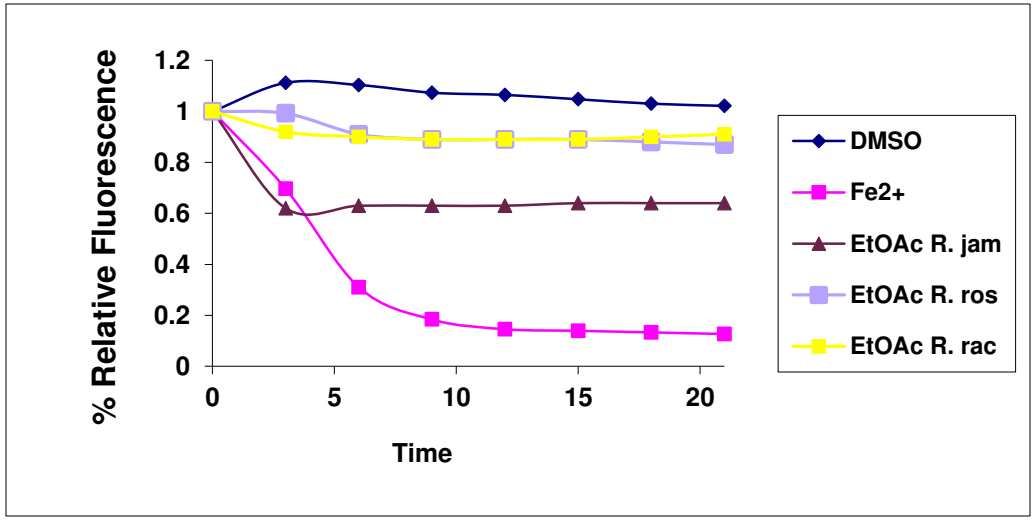


Liposome

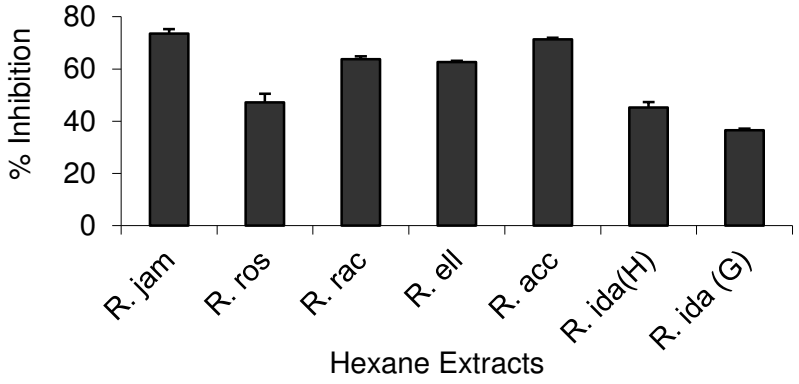
Courtesy of wikipedia.org

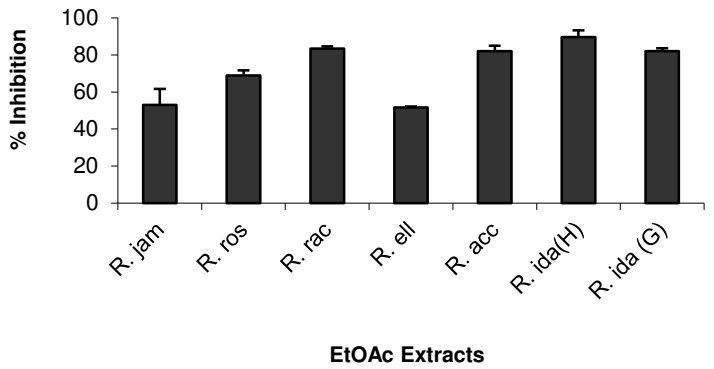
LPO Inhibitory Activity of *Rubus* Extracts (50 µg/mL)

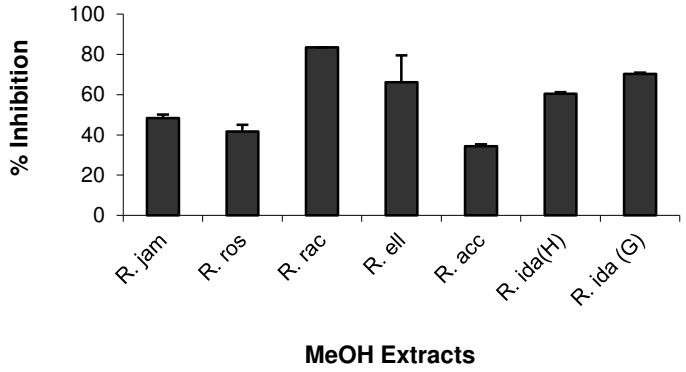




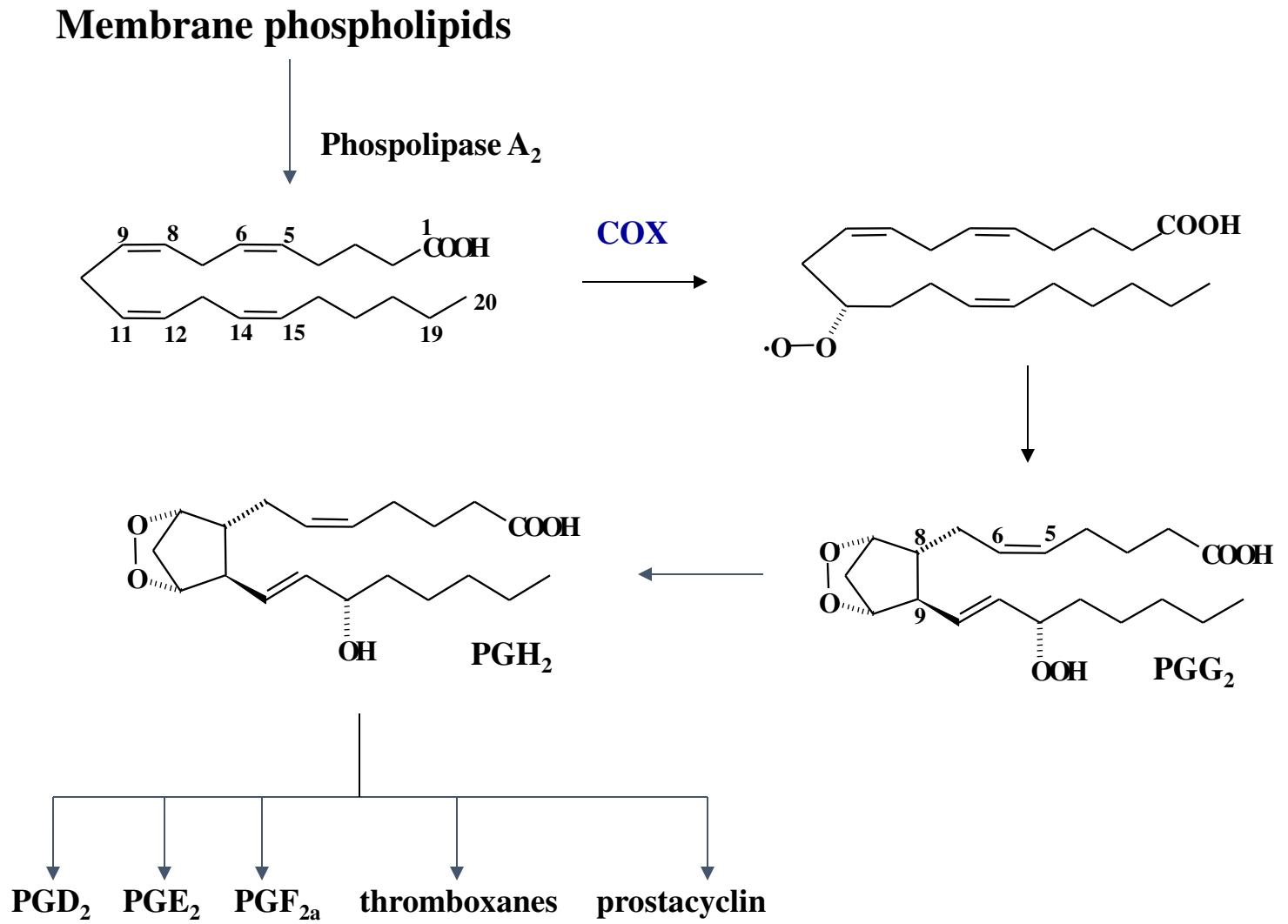
LPO Inhibitory Activity of Extracts (50 ppm)



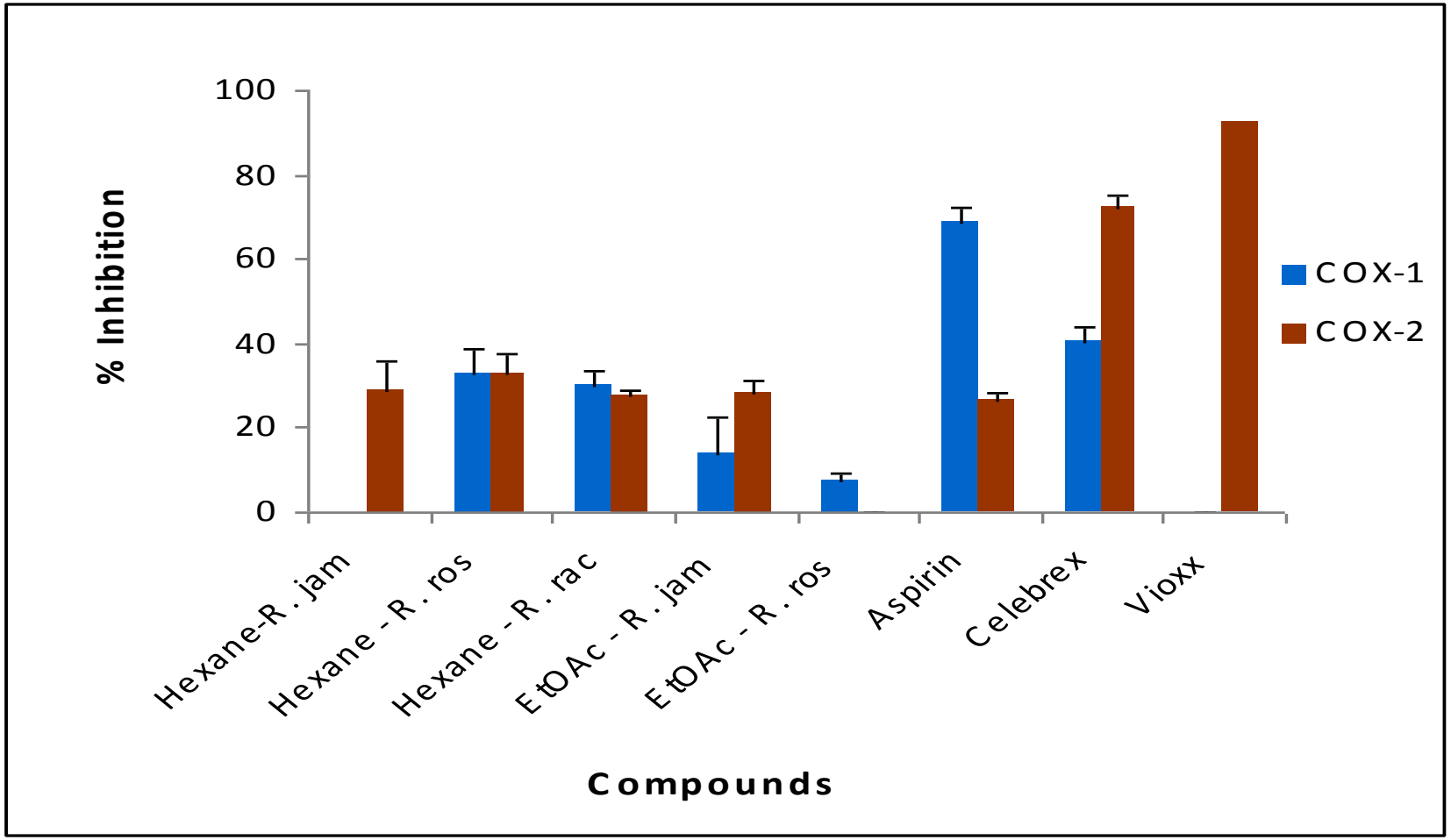




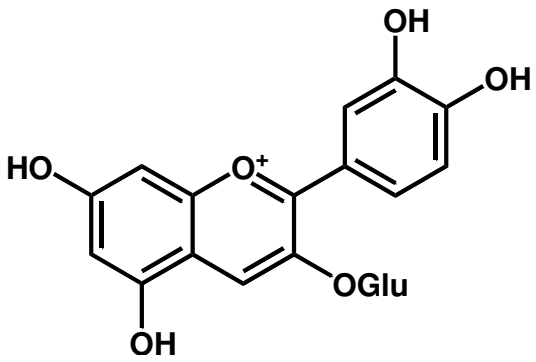
Cyclooxygenase (COX) Enzyme



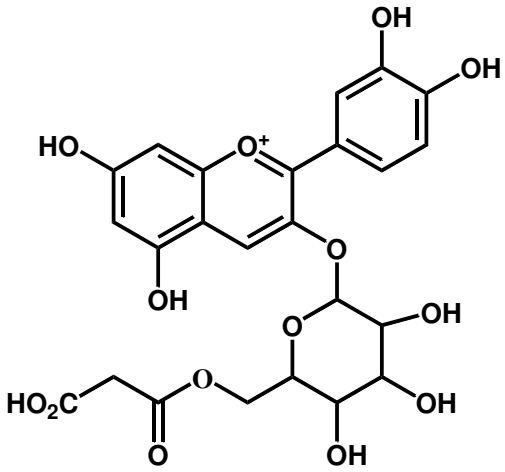
COX Enzyme Inhibitory Activity of Extracts (50 ppm)



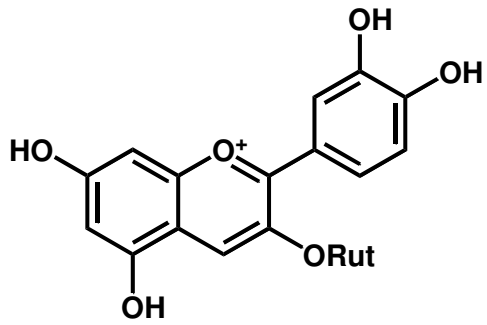
Anthocyanins in the Berries



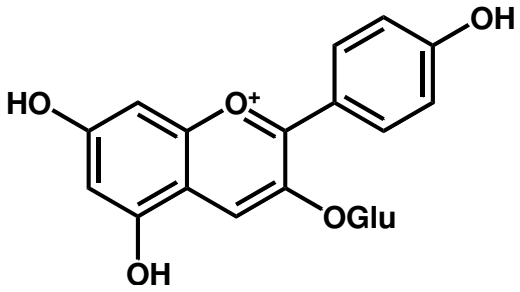
Cyanidin-3-*O*-glucoside: A1



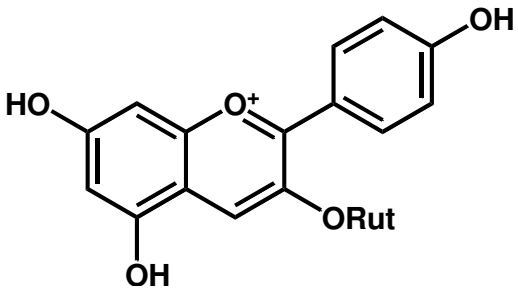
Cyanidin-3-*O* –glucosyl malonate: A5



Cyanidin-3-*O*-rutinoside: A2

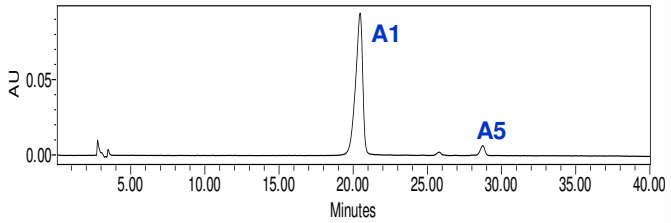


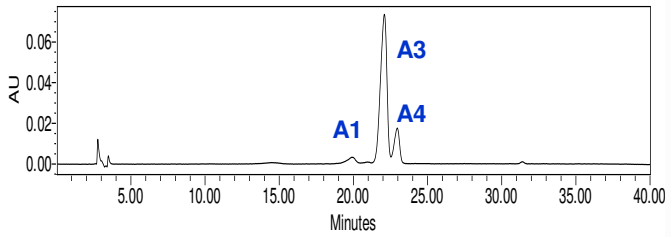
Pelargonidin-3-*O*-glucoside: A3

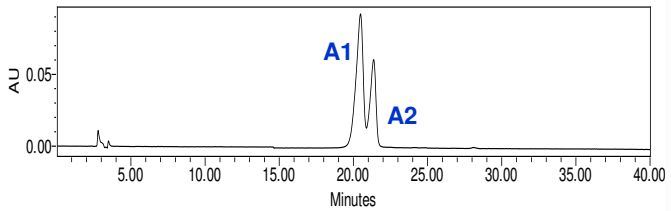


Pelargonidin-3-*O*-rutinoside: A4

Quantification of Anthocyanins

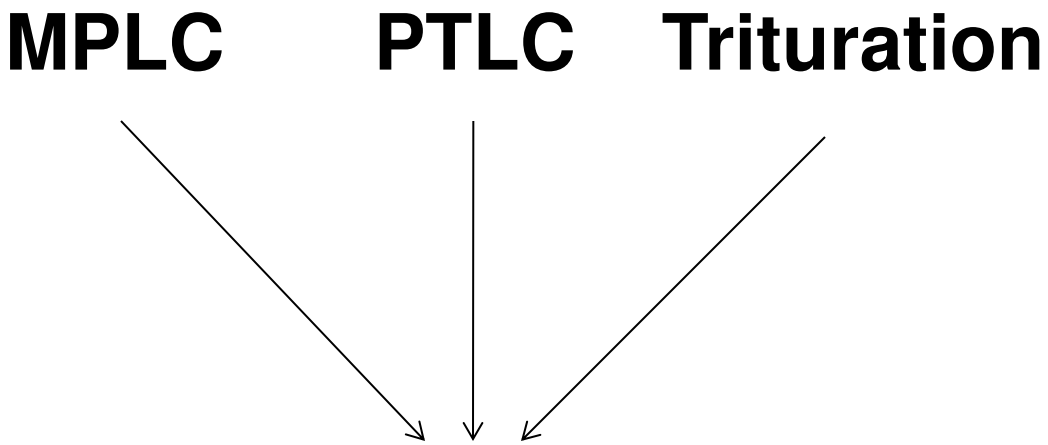




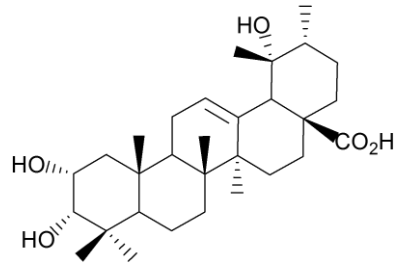


	mg/100g FW	Total (mg/100g FW)
R. jamaicensis		
A1: Cyanidin-3-*O*-glucoside	1501.3	1672.8
A5: Cyanidin-3-*O*-glucosyl-malonate	171.5	
R. rosifolius		
A1: Cyanidin-3-*O*-glucoside	17.1	145.7
A3: Pelargonidin-3-*O*-glucoside	80.6	
A4: Pelargonidin-3-*O*-rutinoside	48	
R. racemosus		
A1: Cyanidin-3-*O*-glucoside	1437	1882
A2: Cyanidin-3-*O*-rutinoside	444.8	
R. accuminatus		***2199***
R. idaeus (H)		***314***

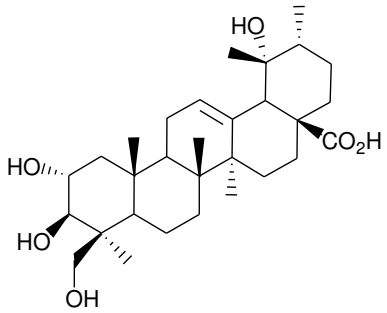
Natural Products from EtOAc Extract of *R. rosifolius*



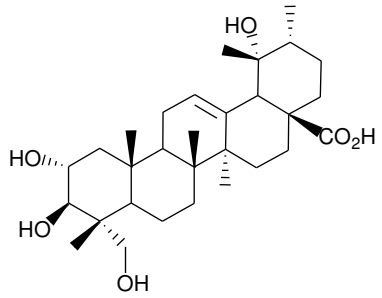
Ursolic Acid Analogues from *R. rosifolius*



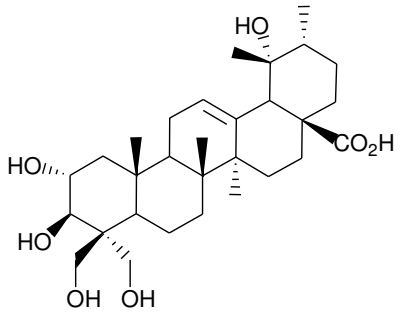
1: Euscafic acid (0.004%)



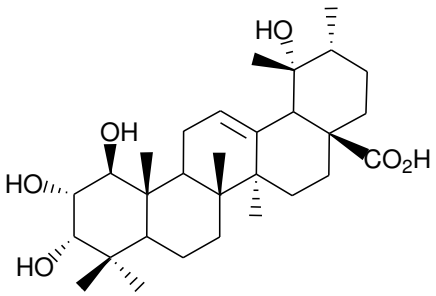
3: Hyptatic acid (0.0046%)



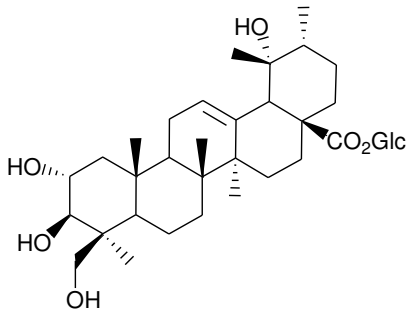
4:19-α-Hydroxyasiatic acid (.0053%)



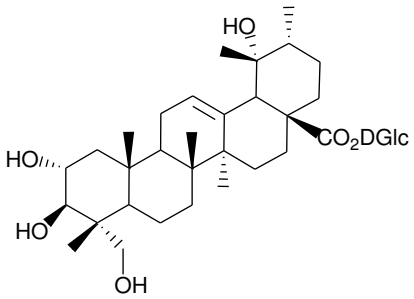
5: Trachelosperogenin B-1 (0.0005%)



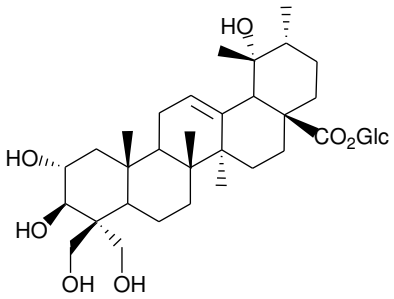
2: 1-β-Hydroxyeuscafic acid (0.002%)



6: 4-epi-Nigaichigoside (0.003%)

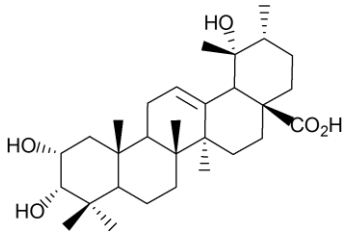
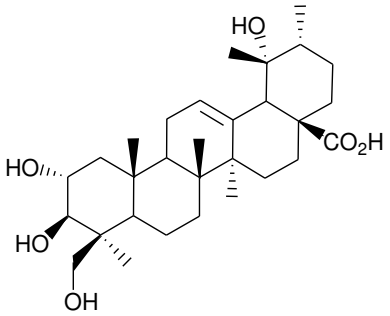
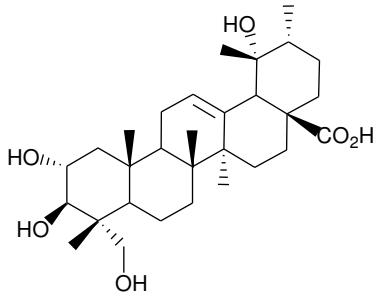
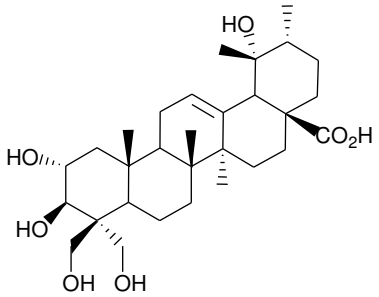
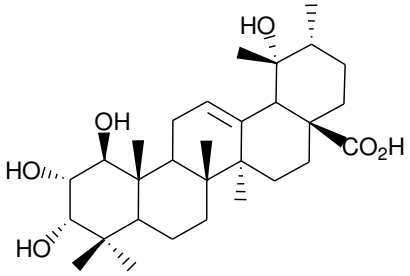
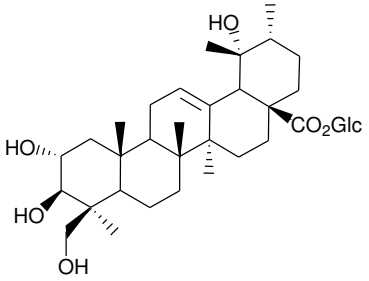
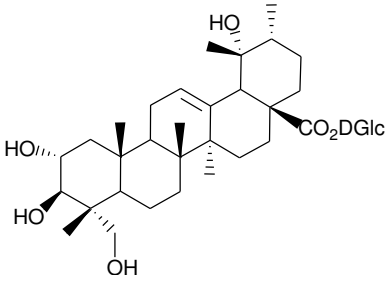
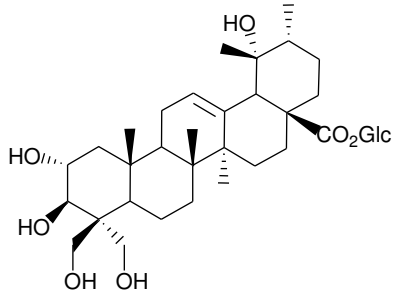


7: Nigaichigoside (0.002%)

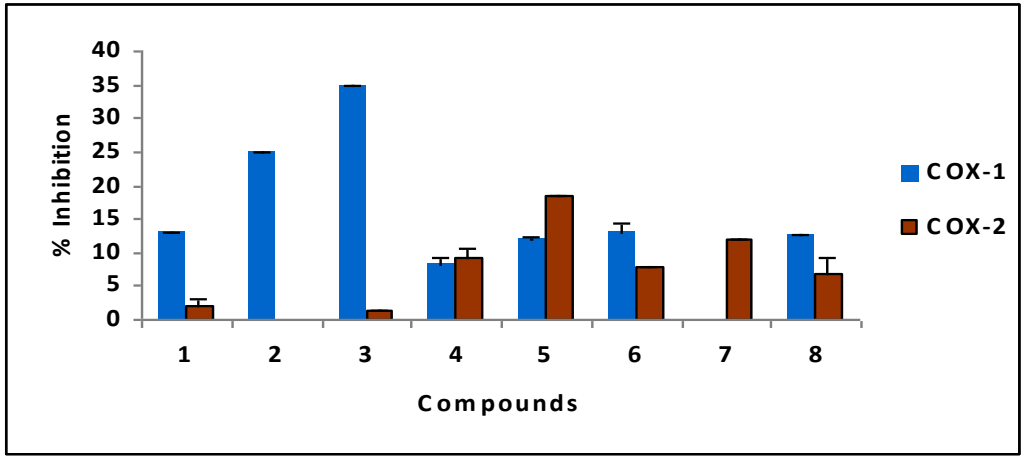


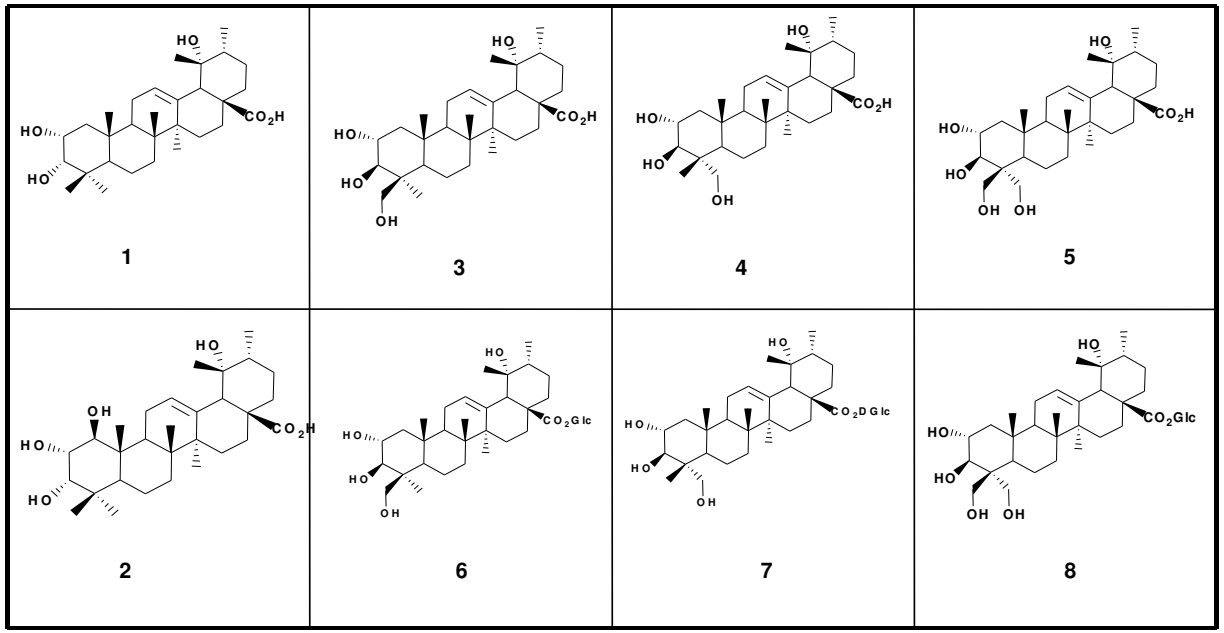
8: Trachelosperoside B-1 (0.0005%)

LPO Inhibitory Activity of Compounds (25 ppm)

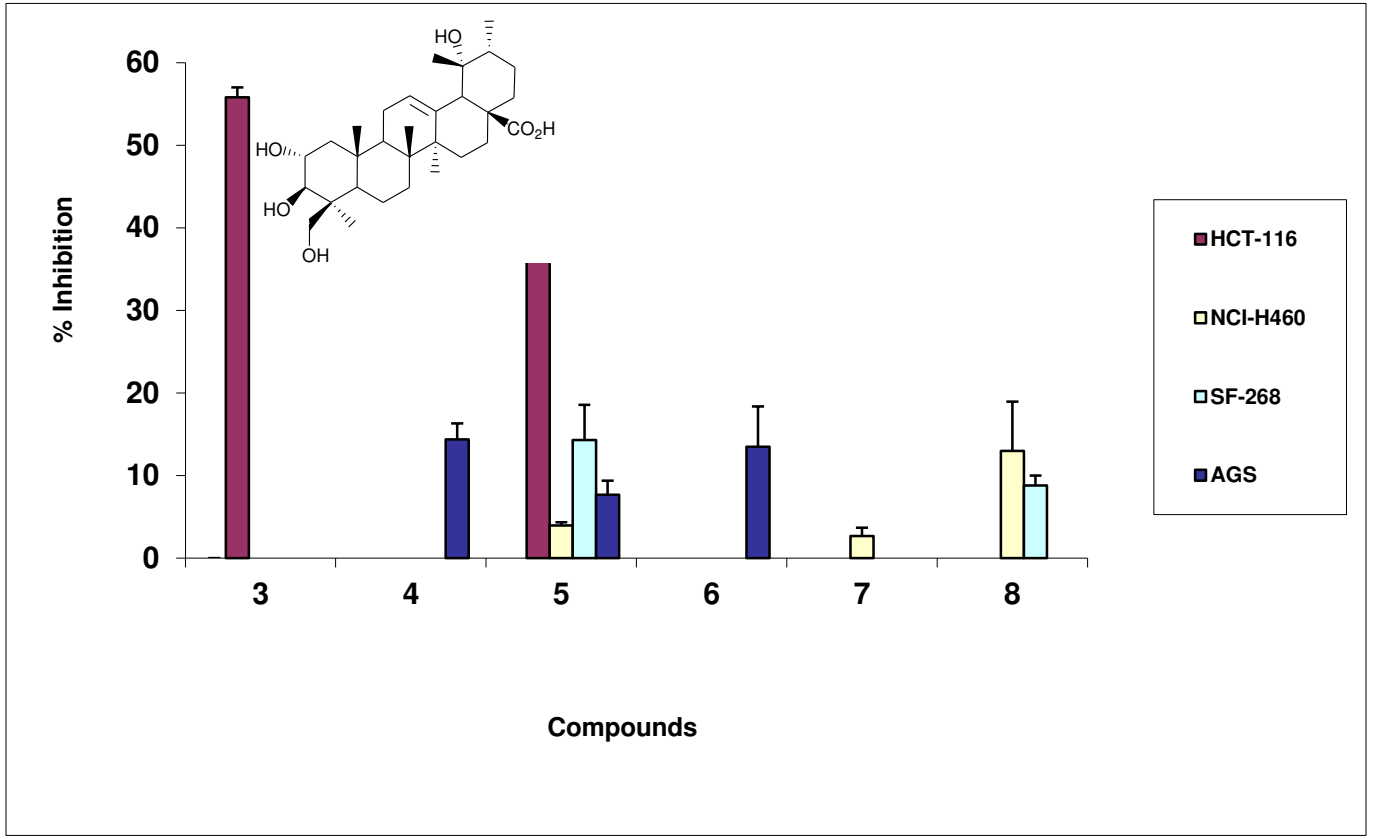
 **1: 33%**	 **3: 37.5%**	 **4: 68%**	 **5: 16%**
 **2: 70.5%**	 **6: 80%**	 **7: 60%**	 **8: 35.5%**

COX Enzyme Inhibitory Activity of Compounds (25 ppm)





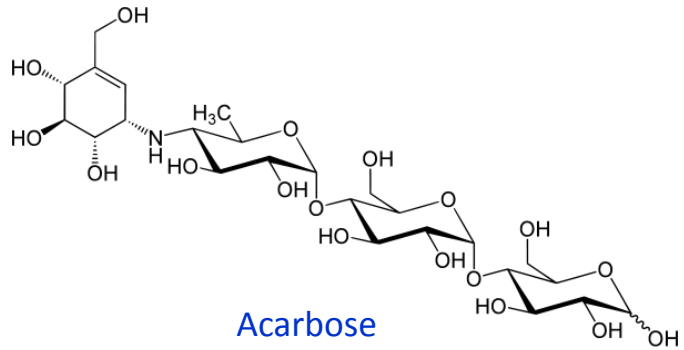
Inhibition of Tumor Cell Proliferation (100 ppm)



colon (HCT-116), lung (NCIH460), central nervous system (SF-268), gastric (AGS)

α-Glucosidase Inhibition by Compounds from *R. ellipticus*

Compound ID	IC_{50} (μg/mL)
78F	245.96 ± 25.64
80D	**50.20** ± 18.12
Acarbose	105 ± 9.02



Acarbose

Glucobay-Europe and China
Precose-USA
Prandage- Canada

Rubus jamaicensis Leaves

Fatty Acid Profile of *R. jamaicensis* Leaves

Retention time/min	Systematic Name	Common Name	% Composition
11.43	Dodecanoic acid	Lauric acid	6.57 ± 2.43
16.72	Tetradecanoic acid	Myristic acid	1.99 ± 0.38
24.46	Hexadecanoic acid	Palmitic acid	34.66 ± 7.77
33.30	all-cis 9,15-Octadecadienoic acid	Linoleic acid	5.75 ± 0.85
33.67	all-cis 9,12,15-Octadecanoic acid	α-Linolenic acid	24.56 ± 3.84
35.23	Octadecanoic acid	Stearic acid	12.29 ± 3.10
48.16	Eicosanoic acid	Arachidic acid	2.21 ± 0.23
61.20	Docosanoic acid	Behenic acid	3.59 ± 2.00

Antimicrobial Activity of *R. jam.* *Leaf* Extracts

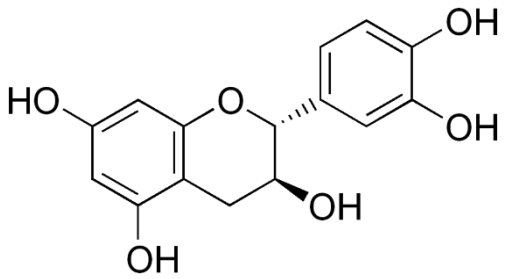
Extract	*E. coli*		*S. aureus*		*S. pyrogenes*		*P. aeruginoss*	
	Zone/mm	MIC/ mg/ml	Zone/mm	MIC/ mg/ml	Zone/mm	MIC/ mg/ml	Zone/mm	MIC/ mg/ml
Hex	-	-	-	-	-	-	-	-
EtoAc	-	-	-	-	8	10.0	9	10.0
MeOH	12	2.5	12	2.5	-	-	12	5.0
BuOH	10	2.5	12	2.5	-	-	14	5.0
H_2O	10	1.3	9	0.3	9	1.3	12	1.3

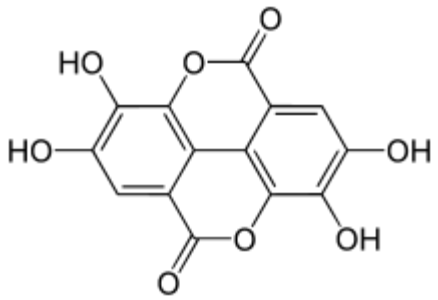
Total Phenolics and Antioxidant Activity of *R. jam.* Extracts

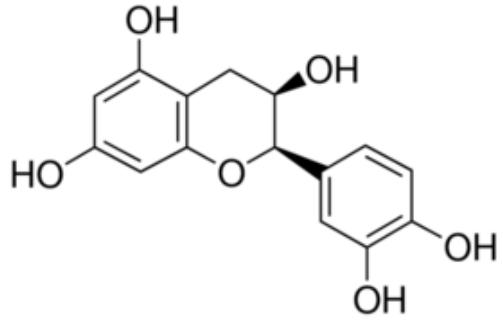
Extract	GAE (mg gallic acid/g D.W)	Radical scavenging activity/%
Hex	0.98 ± 0.01	4.76 ± 0.04
EtOAc	14.65 ± 0.21	53.90 ± 0.73
MeOH	40.08 ± 0.28	84.58 ± 0.58
H_2O	12.68 ± 0.23	81.47 ± 1.36

LC-MS/MS Determination of Phenolics in *R. jam.*

Compound	Phenolic content (mg/Kg D.W)		
	Catechin	Ellagic acid	Epicatechin
	3.48 ± 0.54	30.17 ± 1.40	515.92 ± 26.72
Molecular weight	290.27	302.20	290.37







CYP 450 Inhibitory Activity of *R. jam.* Leaf Extracts

Extract	Average inhibition/ %	
	1A1	1B1
Water	97.2 ± 1.9	86.9 ± 5.1
Fat	88.5 ± 1.1	-

Summary

- Pioneered research on Jamaica's blackberry and raspberry fruit species, and discovered their outstanding health-beneficial properties

- Jamaican berries compare very well with several species of berries commercially produced in the US, with respect to antioxidant, anticancer, anti-inflammatory activities, and anthocyanin contents. In many instances, the Jamaican berries demonstrated superior properties.

- Compound from raspberry species demonstrates antidiabetic activity superior to the commercial drug, Acarbose.

Summary

- Compounds from Jamaican raspberries demonstrate a number of positive biological activities, including antioxidant and anticancer properties against breast, colon, lung, central nervous system and gastric cancer cell lines.

- The oil from *R. jamaicensis* leaves possess 4:1 oemga-3:omega-6 ratio.

- *R. jamaicensis* leaves have demonstrated very good antioxidant, antibacterial, and CYP 450 inhibitory activity.

Acknowledgements

- U.W.I.
- UWI Research Team
- Prof. Muralee Nair
- Dr. Vanisree Mulabagal
- Dr. Yunbao Liu
- Dr. Navindra Seeram- URI
- Mr. Tao Yuan
- Dr. Yanjun Zhang
- Dr. Rupika Delgoda

Thank You!

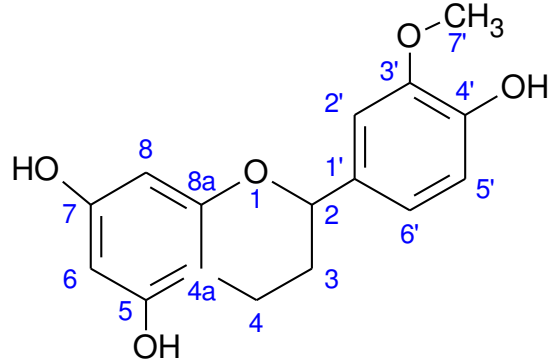
MS extracts	*HCT-116*		*MCF-7*		*CCD-18Co*	
	48 h	*72 h*	*48 h*	*72h*	*48 h*	*72h*
[exane extract	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
/l acetate extract	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ethanol extract	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
67E	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
68M	95.6±2.6[a]	83.7±2.2	89.3±3.3	76.4±2.4	n.d.	132.2±2.6
63C	83.8±1.6	73.7±2.6	79.3±2.1	71.0±1.8	n.d.	107.2±2.8
61B	94.6±1.5	88.1±2.7	90.6±1.8	83.9±3.0	n.d.	119.9±1.1
62A	131.8±2.8	123.8±3.3	n.d.	125.0±1.9	n.d.	n.d.

Value Chain

- Research & Development: Companies research and develop new and improved products like seeds, fertilizer, plant protection products, irrigations systems, and machinery.
- Input distribution & Adoption: Inputs – including seeds, fertilizer, crop protections, irrigation systems, and machinery – are sold to farmers for use in agricultural products, with the purpose of increasing yields.
- Farming: Smallholders grow crops while working with companies to develop new farming systems for local, regional, and international markets.
- Sourcing & Trading: Companies "source" or purchase products from smallholders through different types of arrangements (e.g. contract farming, sharecropping, etc.) and trade them through regional and international markets.
- Processing & Manufacturing: Companies work with smallholders to process raw materials and manufacture products locally.
- Retail & Export: Companies sell food, drinks, fuels, fibers, oils, and other agriculture-based products either to other companies or consumers directly (e.g. supermarkets).

http://www.one.org/us/policy/new-alliance-for-food-security-and-nutrition-part-2/

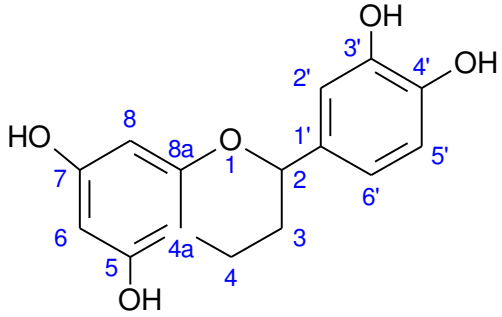
Natural products from R. Jamaicensis



- 3’-O-methyl-catechin

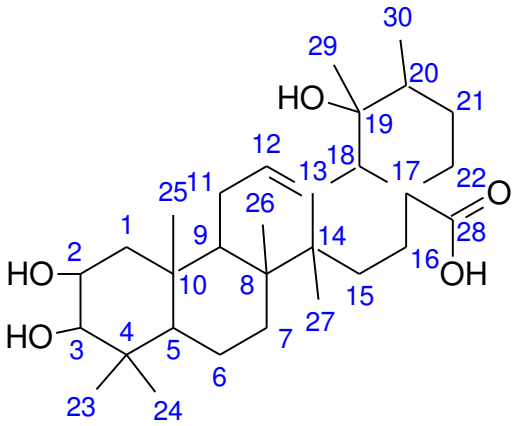
Carbon #	δC	δH	COSY	HMBC	NOSY
2	80.03	4.82 d	4.18	67.63, 29.42, 115.52, 116.15	2.47, 2.87
3	67.63	4.18 q			2.47, 2.87
4	29.42	2.47 s, 2.87 dd	2.47, 2.87	80.03, 67.63, 157.38, 96.12	
4a	100.31				
5	158.13				
6	96.63	5.96s		157.38, 96.12	
7	157.38				
8	96.12	5.94s		158.13, 96.12, 116.15	
8a	157.52				
1’	132.45				
2’	115.52	6.99s	4.18	80.03, 145.93, 119.63	
3’	146.09				
4’	145.93				
5’	116.15	6.78d		145.93	4.18
6’	119.63	6.81	4.18	80.03	4.18
7’	50.05	3.36d	2.47, 2.87		

- Epicatechin



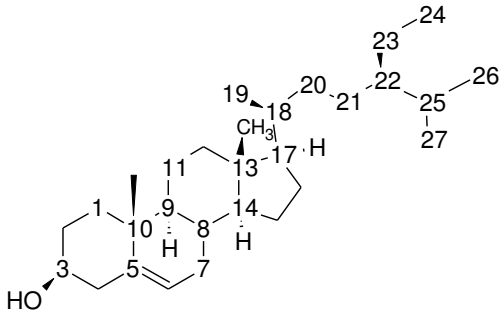
Carbon #	δC	δH	COSY	HMBC
2	79.90	4.817		67.52, 115.46, 119.60, 132.35 , 157.57
3	67.52	4.192	5.24, 4.89	96.1
4	29.35	2.87 dd, 2.75 d	4.19	100.31, 157.42 67.52,79.90, 100.31,157.42
4a	100.31			
5	158.00			
6	96.62	6.243		157.4
7	157.60			
8	96.11	5.964 d		96.62,100.31,157.42, 158.00
8a	157.42			
1’	132.35			
2’	116.16	6.798	4.820	115.46
3’	145.96			
4’	145.80			
5’	115.46	7.002	4.827	79.90, 119.62, 145.80
6’	119.62	6.821	5.173	70.70,132.35, 145.80, 115.46

- Euscaphic acid



Carbon#	δC	δH	COSY	HMBC
1	42.49	1.575	3.930	
2	67.11	3.939		
3	80.11	3.341	3.970	48.21,67.11,39.55,41.20
4	39.55	0.885		
5	48.21	1.871		
6	19.48	1.466		
7	34.02	1.590	1.314	
8	41.20			
9	49.35	1.279		
10	39.55	0.885		
11	24.92	1.360	3.923	
12	129.36	5.310	2.506	42.74,48.21,55.08
13	140.07			
14	42.74	1.249		67.11,41.20
15	29.58	1.799		
16	27.29	1.731		
17	49.20			
18	55.08		1.002,1.518,1.819	129.36,140.07,48.21,182.28,43.09
19	73.59			
20	43.09	1.365	3.920	140.07,42.74,29.58,41.20
21	26.61	1.515	1.030,1.517,1.840	
22	39.02	1.736		
23	29.24	1.008	1.827	
24	16.78			
25	16.90	0.999		
26	17.60	0.793		42.30,73.59
27	24.71	2.019	5.290	
28	182.28			
29	27.07	1.207	2.879	73.59,55.08,43.09,140.07
30	16.60	0.949		

- ß-sitosterol



Carbon#	δC	δH	COSY	HMBC
1	37.25	1.852	1.085,1.269,1.523	
2	31.66	1.501	1.986	
3	71.80	3.500	1.502,1.839,2.271	14.6
4	42.31	2.323	1.841	36.50,71.80,121.70,140.75
5	140.75			
6	121.70	5.351	2.221,2.283,1.975,1.511	24.29,31.90
7	31.90	1.833		
8	**31.9**			
9	50.13	0.945		33.94,36.13,56.05
10	36.50	1.109	1.862	
11	21.08	1.509	1.958	
12	39.77	2.036	1.158	
13	**42**			
14	56.76	1.014	1.858	36.50,50.13
15	26.09	1.152		
16	**28.23**			
17	56.05	1.105	1.837	
18	**36.13**			
19	19.02			
20	33.94	1.983	1.510	
21	**26.09**			
22	45.84	0.951		
23	23.07	1.299		
24	11.97			
25	29.15	1.647	0.828	
26	19.80	0.835		29.15,45.84
27	19.38			
28	18.76	0.68		
29	11.85	1.01		

Fatty acid profile of EtoAc extract

Fatty Acid	Percentage FA/%
Lauric	2.42 ± 0.39
Myristic	1.14 ± 0.09
Palmitic	22.21 ± 1.58
Margaric	0.39 ± 0.03
Stearic	11.13 ± 0.71
α-Linolenic	33.30 ± 3.70
Linoleic	10.29 ± 1.00
Arachidic	2.52 ± 0.24
Behenic	3.29 ± 1.22
Lignoric	0.64 ± 0.22

Figure 11: Fats present in derivatized EtoAc extract

Though Cyp 450 enzymes perform vital roles within biological systems, they are linked to the production of procarcinogens and ROS.[17] Their inhibition in biological systems therefore have anticancer activity implications. As shown in **Table 3.7,** the butanol partition of the polar aqueous extract exhibit the highest inhibition of 97.2% against the 1A1 which typically interacts with procarcinogens such as polyaromatic hydrocarbons (PAHs) and environmental toxins such as dioxins.[18]