



Zambroza - Continues its Leadership Position in the Mangosteen Supplement Market...

THREE NEW XANTHONES DISCOVERED!!!

Researchers from Ohio State University and Nature's Sunshine Products have jointly announced **the discovery of three new xanthones**, with a paper presented to the American Society of Pharmacognosy. The pharmacognosy department at Ohio State is

heavily involved in looking at anti-cancer potential of many natural substances, including mangosteen and sea buckthorn, BOTH of which are in Zambroza™. Note as you take a look at the paper presented by Ohio State, the pictures of the mangosteen tree, the mangosteen fruit, and of course, a bottle of Zambroza™ mangosteen juice! As you can read from the paper, Ohio State is excited about the possible benefits of two of these xanthones in particular. A one-page abstract of the paper detailing the discovery of these two new xanthones can be read here, as well as an abstract for their work about sea buckthorn. Sea Buckthorn is Sibujice's claim to fame and present in Zambroza as well! Another new drink on the market is Goji, which contains powerful Wolfberries, also ... you guessed it ... found in Zambroza too!!! We've really got it all!

Questions and answers concerning this study:

1. Do any other mangosteen products contain sea buckthorn?

To our knowledge, the answer is no. Nature's Sunshine spent over a year testing and researching the best ingredients and the right forms to use in Zambroza™. However, we are sure that others will try to copy our success in the future.

2. Do any other mangosteen products contain these new xanthones?

It is very probable that the other drinks (Xango™, MangoXan™, Vemma™, etc.) contain small trace amounts of these xanthones. After all, they are in the mangosteen fruit--and if these companies don't destroy these xanthones by their processing (which is definitely NOT a sure thing), then there may be very small amounts there. However, Nature's Sunshine uses a proprietary extraction process that can pull these xanthones out of the mangosteen rind and concentrate them into the drink at a high level. Xango™ CANNOT accomplish this by their method of simply throwing the rind in and hoping for the best. They are stuck with what they get, even before they dilute their juice and heat it.

3. What further research is being done?

That is up to the scientists, but knowing their affiliation and passion for pharmacognosy, it is safe to say that they will be looking closer.



ANTIOXIDANT XANTHONES FROM THE PERICARP OF *GARCINIA MANGOSTANA* (MANGOSTEEN)

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Introduction

Garcinia mangostana L. (Guttiferae), commonly known as "Mangosteen" (Figure 1), is one of the most widely recognized tropical fruits and has great appeal because of its quality of color, shape, and flavor. In Thai folk medicine, the pericarp of Mangosteen has been used for many years to healing skin infections and wounds and for relief of diarrhea. The antioxidant and anticancer activities of an extract of the pericarp of Mangosteen have been reported.¹ Mangosteen is used as a constituent of an herbal remedy sold in the United States for its antioxidant effects. Previously phytochemical investigations have indicated that the major components of Mangosteen are prenylated xanthenes.²

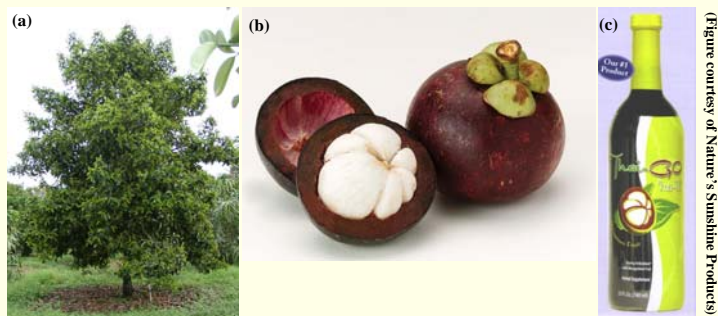


Figure 1. (a) Whole tree; (b) Fruit; (c) Product containing *G. mangostana*.

Isolation and Identification of Compounds

As part of our ongoing research on cancer chemopreventive agents from botanical dietary supplements, the pericarp of Mangosteen was selected for detailed studies. Repeated chromatography of the CH₂Cl₂-soluble extract led to the isolation of three new highly oxygenated prenylated xanthenes, 8-hydroxycudraxanthone G (1), 7-methoxy-2-(3-methyl-2-butenyl)-8-(3-methyl-2-oxo-3-butenyl)-1,3,6-trihydroxyxanthone (2) and 8-hydroxymangostinone (3), along with seven known xanthenes, α -mangostin (4), γ -mangostin (5), 8-deoxygartanin (6), gartanin (7), mangostinone (8), tovophyllin A (9), and cudraxanthone G (10) (Figure 2). The structures of all isolates were elucidated by spectroscopic data analysis as well as by comparison with published data.

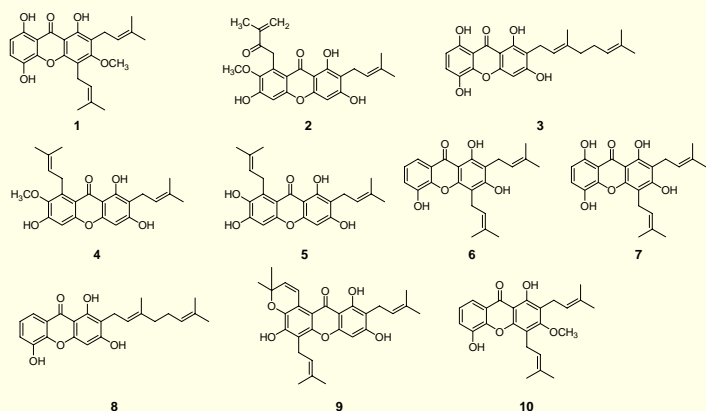


Figure 2. Structures of compounds 1-10 from *G. mangostana*.

8-Hydroxycudraxanthone G (1) Yellow solid. UV (MeOH) λ_{\max} nm (log ϵ): 238 (4.28), 263 (4.38), 279 (4.34), 351(3.97) nm. IR (dried film) ν_{\max} 1623, 1584, 1490, 1217, 1098 cm⁻¹. HRESIMS m/z 433.16114 [M+Na]⁺ (calcd for C₂₄H₂₆O₆Na⁺, 433.16216). ¹H NMR (300 MHz, CDCl₃) δ 12.22 (OH), 11.22 (OH), 7.25 (1H, d, *J* = 9.0 Hz, H-6), 6.69 (1H, d, *J* = 9.0 Hz, H-7), 5.24 (2H, m, H-2' and H-2''), 3.81 (3H, s, 3-OCH₃), 3.54 (2H, d, *J* = 6.2 Hz, H-1'), 3.41 (2H, d, *J* = 6.9 Hz, H-1''), 1.87 (3H, s, H-5'), 1.81 (3H, s, H-5''), 1.74 (3H, s, H-4'), 1.71 (3H, s, H-4''). ¹³C NMR (75 MHz, CDCl₃) δ 185.4 (C-9), 164.4 (C-3), 158.8 (C-1), 153.8 (C-8), 152.7 (C-4a), 142.8 (C-10a), 135.9 (C-5), 132.3 (C-3'), 132.2(C-3''), 123.0 (C-6), 123.0 (C-2''), 122.2 (C-2'), 118.2 (C-2), 113.0(C-4), 109.8 (C-7), 107.3 (C-8a), 104.9 (C-9a), 62.1 (3-OCH₃), 25.7 (C-4'), 25.5 (C-4''), 23.0 (C-1'), 22.5 (C-1''), 18.0 and 17.9 (C-5' or C-5'').³

7-Methoxy-2-(3-methyl-2-butenyl)-8-(3-methyl-2-oxo-3-butenyl)-1,3,6-trihydroxyxanthone (2)

Yellow solid. UV (MeOH) λ_{\max} nm (log ϵ) 243 (3.84), 320 (3.65), 354 (3.32) nm. IR (dried film) ν_{\max} 1608, 1578, 1465, 1284, 1162, 1081 cm⁻¹. HRESIMS m/z 447.14323 [M+Na]⁺ (calcd for C₂₅H₂₄O₆Na⁺, 447.14142). ¹H NMR (300 MHz, acetone-*d*₆) δ 13.50 (OH), 6.86 (1H, s, H-5), 6.39 (1H, s, H-4), 6.23 (1H, s, H-4'a), 5.86 (1H, s, H-4'b), 5.24 (1H, t, H-2'), 4.75 (2H, s, H-1''), 3.73 (3H, s, 3-OCH₃), 3.30 (2H, d, *J* = 7.2 Hz, H-1'), 1.92 (3H, s, H-5'), 1.75 (3H, s, H-4'), 1.61 (3H, s, H-4''). ¹³C NMR (75 MHz, acetone-*d*₆) δ 199.1 (C-2''), 182.2 (C-9), 163.3 (C-3), 161.4 (C-1), 161.1 (C-6), 156.1 (C-4a), 155.8 (C-10a), 145.8 (C-3'), 145.7 (C-7), 131.4 (C-8a), 131.2 (C-3''), 123.6 (C-2'), 123.6 (C-4''), 111.0 (C-8a), 111.0 (C-2), 103.3 (C-5), 103.2 (C-9a), 93.4 (C-4), 61.3 (3-OCH₃), 37.9 (C-1'), 25.9 (C-4'), 22.0 (C-1''), 18.1 (C-5'), 17.9 (C-5'').⁴

8-Hydroxymangostinone (3)

Yellow solid. UV (CHCl₃) λ_{\max} nm (log ϵ) 243 (3.84), 255 (3.86), 281 (3.18), 341 (3.60) nm. IR (dried film) ν_{\max} 1634, 1592, 1505, 1447, 1253, 1086, 1054 cm⁻¹. HRESIMS m/z 419.14466 [M+Na]⁺ (calcd for C₂₃H₂₄O₆Na⁺, 419.14651). ¹H NMR (300 MHz, acetone-*d*₆) δ 12.30 (OH), 11.25 (OH), 7.27 (1H, d, *J* = 8.7 Hz, H-5), 6.61 (1H, d, *J* = 8.7 Hz, H-6), 6.57 (1H, s, H-4), 5.29 (1H, t, 6.3, H-2'), 5.06 (1H, t, 6.9, H-2''), 3.37 (2H, d, *J* = 7.2 Hz, H-1'), 2.04 (2H, m, H-1''), 1.96 (2H, t, 7.5, H-4'), 1.79 (3H, s, H-5'), 1.59 (3H, s, H-4''), 1.54 (3H, s, H-5''). ¹³C NMR (75 MHz, acetone-*d*₆) δ 185.4 (C-9), 165.0 (C-3), 160.9 (C-1), 156.6 (C-4a), 154.1 (C-8), 144.5 (C-10a), 137.9 (C-5), 135.5 (C-3'), 131.6 (C-3''), 125.1 (C-2''), 124.3 (C-6), 122.9 (C-2'), 112.1 (C-2), 110.2 (C-7), 108.5 (C-8a), 102.5 (C-9a), 94.6 (C-4), 40.5 (C-4'), 27.4 (C-1''), 25.8 (C-4''), 21.8 (C-1'), 17.7 (C-5''), 16.2 (C-5').⁵

Antioxidant Activity Evaluation

Measurement of the ONOO⁻ Scavenging Activity

DL-Penicillamine (DL-2-amino-3-mercapto-3-methylbutanoic acid) were purchased from Sigma Chemical Company (St. Louis, MO). DHR 123 (dihydrorhodamine 123) was purchased from Molecular Probes (Eugene, OR), and ONOO⁻ and morpholinosydnonimine (SIN-1) were purchased from Cayman Chemicals Co. (Ann Arbor, MI).

The ONOO⁻ scavenging activity was measured by monitoring the oxidation of DHR 123, by modification of the method of Kooy et al.⁶ In brief, DHR 123 (5 mM) in dimethylformamide, purged with nitrogen, was stored at -80°C as a stock solution. The rhodamine buffer used consisted of 90 mM sodium chloride, 50 mM sodium phosphate dibasic, 50 mM sodium phosphate monobasic, 5 mM potassium chloride at pH 7.4, and 100 μ M diethylenetriaminepentaacetic acid (DTPA), each of which was prepared with high quality deionized water and purged with nitrogen. The final concentration of DHR 123 was 5 μ M by dilution of this buffer. The background and final fluorescent intensities of oxidized DHR 123 were measured at 5 min after treatment with and without the addition of authentic ONOO⁻ and SIN-1 derived ONOO⁻ (10 μ M) by a microplate fluorescence reader FL 500 (Bio-Tek Instruments Inc.) at the excitation and emission wavelengths of 480 and 530 nm, respectively. Results were expressed as the mean (n = 3) for the final fluorescence intensity minus background fluorescence. DL-Penicillamine was used as a positive control.

Results

Peroxyntirite (ONOO⁻), generated from nitric oxide (NO⁻) and superoxide anion (⁻O₂) in vivo, has been reported to act as an oxidizing and nitrating agents and involve in the initiation of carcinogenesis, along with NO⁻.

Except for compound 2, which was isolated as a very minor compound, the antioxidant activities of the other nine isolates (1, and 3-10) obtained in the present study were determined by using authentic and morpholinosydnonimine (SIN-1) derived ONOO⁻. Five compounds (1, 3, 4, 5 and 7) were demonstrated to possess pronounced antioxidant activity in the assays utilized (Table 1).

Table 1. Antioxidant activities of isolates from *G. mangostana* on ONOO⁻ assays

Compounds	IC ₅₀ (μ M) ^a	
	Authentic ONOO ⁻	SIN-1 derived ONOO ⁻
1	4.61	10.03
3	2.20	9.65
4	12.20	<0.49
5	7.95	3.14
6	N.A. ^c	11.90
7	9.09	9.27
8	N.A.	N.A.
9	N.A.	N.A.
10	N.A.	3.20
DL-penicillamine ^b	3.14	7.35

^aONOO⁻ is the scavenging activity of authentic peroxyntirite and generated peroxyntirite from SIN-1 (IC₅₀ μ M). Values of ONOO⁻ scavenging/inhibitory activities expressed as the mean of three experiments. ^bDL-penicillamine used as positive control. ^cN.A.: no activity within the tested concentration (5-100 μ M).

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References

- Primchanien, M.; Nuttavut, K.; Sineenart, K.; Omboon, L.; Narongchai, P.; Neelobol, N. *J. Ethnopharmacol.* **2004**, *90*, 161-166.
- Peres, V.; Nagem, T. J.; Faustino de Oliveira, F. *Phytochemistry* **2000**, *55*, 683-710.
- Hano, Y.; Matsumoto, Y.; Sun, J.-Y.; Nomura, T. *Planta Med.* **1990**, *56*, 399-402.
- Nilar, L.J. H. *Phytochemistry* **2002**, *60*, 541-548.
- Asai, F.; Tosa, H.; Tanaka, T.; Iinuma, M. *Phytochemistry* **1995**, *39*, 943-944.
- Kooy, N. W.; Royall, J. A.; Ischiropoulos, H.; Beckman, J. S. *Free Radic. Biol. Med.*, **1994**, *16*, 149-156.