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Article

Phytochemical, Antimicrobial and Antiprotozoal Evaluation of *Garcinia Mangostana* Pericarp and α-Mangostin, Its Major Xanthone Derivative

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Abstract: Five xanthone derivatives and one flavanol were isolated from the dichloromethane extract of *Garcinia mangostana.* Dichloromethane, ethyl acetate extract and the major xanthone (α-mangostin) were evaluated *in vitro* against erythrocytic schizonts of *Plasmodium falciparum*, intracellular amastigotes of *Leishmania infantum* and *Trypanosoma cruzi* and free trypomastigotes of *T. brucei.* The major constituent α-mangostin was also checked for antimicrobial potential against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillius subtilis*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *M. cheleneoi*, *M. xenopi* and *M. intracellulare*. Activity against

P. falciparum (IC_{50} 2.7 μg/mL) and *T. brucei* (IC_{50} 0.5 μg/mL) were observed for the dichloromethane extract, however, with only moderate selectivity was seen based on a parallel cytotoxicity evaluation on MRC-5 cells (IC_{50} 9.4 μg/mL). The ethyl acetate extract was inactive (IC_{50} > 30 μg/mL). The major constituent α-mangostin showed rather high cytotoxicity (IC_{50} 7.5 μM) and a broad but non-selective antiprotozoal and antimicrobial activity profile. This *in vitro* study endorses that the antiprotozoal and antimicrobial potential of prenylated xanthones is non-conclusive in view of the low level of selectivity.

Keywords: *Garcinia mangostana*; α-mangostin; *in vitro*; antiplasmodial; antileishmanial; antitrypanosomal

1. Introduction

The genus *Garcinia* (Guttiferae, syn. Clusiaceae) contains well-known fruit trees with about 35 genera and up to 800 species of which the fruits of many are edible and serve as a substitute for tamarinds in curries [1]. *Garcinia mangostana* Linn., known as mangosteen, is cultivated in the tropical rainforest of Southeast Asian nations like Indonesia, Malaysia, Sri Lanka, Philippines and Thailand where traditional medicine uses the pericarp for the treatment of abdominal pain, diarrhea, cystitis, eczema, dysentery, wound suppuration and chronic ulcers [2,3]. *In vitro* and *in vivo* laboratory studies have demonstrated that extracts of *G. mangostana* have very diverse pharmacological activities including anti-inflammatory, cytotoxic, antioxidant, antitumoral, immunomodulatory, neuroprotective, anti-allergic, antibacterial and antiviral properties [4–7]. Phytochemical investigation of the pericarp of *G. mangostana* revealed the presence of prenylated xanthones, benzophenones, bioflavonoids and triterpenes [8–10]. Over 68 xanthone-type constituents were reported [11], of which the prenylated cage-type is particularly encouraging for further biological and chemical studies. The most studied xanthones are the α-, β-, and γ-mangostins, garcinone E, 8-deoxygartanin and gartanin [7,12].

The present study evaluated the *in vitro* antileishmanial, antiplasmodial and antitrypanosomal potential of the dichloromethane and ethyl acetate extracts of *G. mangostana*, as well as the isolation and characterization of its xanthone constituents.

2. Results and Discussion

2.1. Phytochemical Study

Chromatographic separation and purification of the dichloromethane extract of *G. mangostana* pericarp produced the compounds **1**–**6** (Figure 1). NMR-data (Tables 1 and 2) and comparison with reported data led to the identification of α-mangostin (**1**) [13], β-mangostin (**2**) [14], 1-hydroxy-3,6,7-trimethoxy-2,8-bis (3-methylbut-2-enyl) xanthone (**3**) [15], 9-hydroxycalabaxanthone (**4**) [16,17], tovophyllin A (**5**) [18,19] and catechin (**6**) [20]. α-Mangostin was the major compound isolated from these series, enabling *in vitro* antiprotozoal and antimicrobial evaluation.

Figure 1. Structures of compounds **1**–**6**.

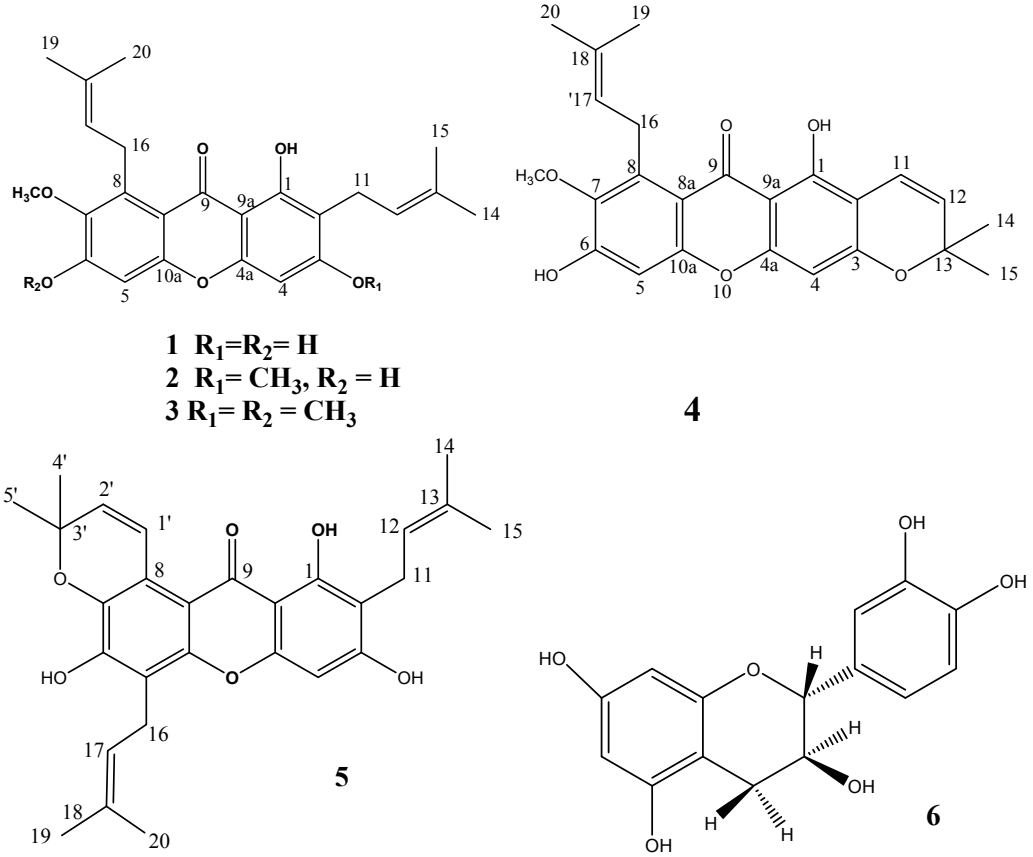

Table 1. [1]H-NMR (500 MHz) spectral data of xanthones **1**–**5**.

Position	Compound 1 [1]	Compound 2 [2]	Compound 3 [2]	Compound 4 [2]	Compound 5 [2]
1	13.72, *s*	13.42, *s*	13.44, *s*	13.72, *s*	13.79, *s*
4	6.28, s	6.24, s	6.30, *s*	6.26, *s*	6.37, *s*
5	6.80, *s*	6.74, *s*	6.75, *s*	6.85, *s*	–
11	3.35, *d* (*J* = 7.3 Hz)	3.37, *d* (*J* = 7.2 Hz)	3.36, *d* (*J* = 7.1 Hz)	6.74, *d* (*J* = 10.0 Hz)	3.48, *d* (*J* = 6.0 Hz)
12	5.17, *t* (*J* = 7.3 Hz)	5.17, *t* (*J* = 7.2 Hz)	5.26, *t* (*J* = 7.1 Hz)	5.58, *d* (*J* = 10.0 Hz)	5.31, *t* (*J* = 7.0 Hz)
14	1.77, *s*	1.75, *s*	1.70, *s*	1.27, *s*	1.79, *s*
15	1.63, *s*	1.62, *s*	1.71, *s*	1.28, *s*	1.71, *s*
16	4.04, *d* (*J* = 7.0 Hz)	4.09, *d* (*J* = 7.2 Hz)	4.15, *d* (*J* = 7.2, Hz)	4.10, *d* (*J* = 7.0 Hz)	3.59, *d* (*J* = 6.0 Hz)
17	5.17, *t* (*J* = 7.3 Hz)	5.18, *t* (*J* = 7.2 Hz)	5.26, *t* (*J* = 7.2 Hz)	5.27, *t* (*J* = 7.3 Hz)	5.31, *t* (*J* = 7.0 Hz)
19	1.71, *s*	1.61, *s*	1.70, *s*	1.71, *s*	1.87, *s*
20	1.73, *s*	1.72, *s*	1.82, *s*	1.82, *s*	1.89, *s*
3-OMe		3.82, *s*	3.91, *s*		
6-OMe			3.97, *s*		
7-OMe	3.71, *s*	3.80, *s*	3.82, *s*	3.83, *s*	
1'					8.00, *d* (*J* = 10.0 Hz)
2'					5.79, *d* (*J* = 10.0 Hz)
4'					1.51, *s*
5'					1.51, *s*

[1] DMSO-d_6, [2] $CDCl_3$.

Table 2. ^{13}C-NMR (125 MHz) spectral data of xanthones **1**–**5**.

Position	Compound 1 [1]	Compound 2 [2]	Compound 3 [2]	Compound 4 [2]	Compound 5 [2]
1	159.9	159.7	157.9	157.8	160.44
2	109.9	111.5	109.6	104.4	108.4
3	162.3	163.5	161.5	159.8	161.6
4	92.3	88.8	86.7	94.0	93.4
4a	154.5	154.4	153.4	156.1	155.3
5	101.8	101.5	96.3	101.6	115.2
6	156.6	155.6	156.1	154.5	151.0
7	143.4	142.5	142.1	142.7	135.8
8	136.3	137.0	135.2	136.9	136.5
8a	112.2	112.3	112.9	112.1	
9	181.3	181.9	180.0	181.8	182.9
9a	103.6	103.8	102.0	103.6	117.2
10a	154.1	155.2	153.4	155.6	
11	21.3	21.3	20.4	115.6	21.4
12	122.4	122.3	121.6	126.9	121.4
13	130.3	132.0	129.6	77.8	132.6
14	25.5	25.8	25.8	28.3	25.8
15	17.9	18.2	16.3	25.6?	17.9
16	25.6	31.2	24.1	26.5	22.6
17	123.7	123.2	120.5	123.1	121.0
18	130.3	131.7	129.7	131.8	131.3
19	17.7	17.8	15.8	18.1	17.9
20	25.7	26.7	24.1	?	25.8
3-OMe	–	55.8	54.9		
6-OMe	–	–	53.9		
7-OMe	60.1	62.0	60.9		
8-OMe				7-OMe	
1'					121.0
2'					131.3
3'					77.1
4'					27.4
5'					27.4

[1] DMSO-d_6, [2] $CDCl_3$.

2.2. In Vitro Antiprotozoal and Antimicrobial Activity

The dichloromethane and ethyl acetate extracts of *G. mangostana* were evaluated in an integrated *in vitro* screen for their antiplasmodial, antileishmanial and antitrypanosomal potential (Table 3). While the ethyl acetate extract showed no antiprotozoal activity at all, a pronounced inhibitory effect (IC_{50}) was obtained with the dichloromethane extract against *Plasmodium falciparum* (IC_{50} 2.7 µg/mL) and *Trypanosoma brucei* (IC_{50} 0.5 µg/mL), but only with acceptable selectivity (SI) for *T. brucei* (SI 18.8). Some side activity was also noted against *T. cruzi* and *Leishmania infantum* (IC_{50} 7.6 and 7.5 µg/mL), but with low selectivity.

The major constituent α-mangostin was also checked for antimicrobial potential against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillius subtilis*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *M. cheleneoi*, *M. xenopi* and *M. intracellulare* (Table 4). Although inhibitory activity could be indicated against *B. subtilis* and *S. aureus* (MIC 1.6 and 3.2 μg/mL) and the *Mycobacterium* species (MIC 1.5 μg/mL), selectivity was quite low in view of the observed cytotoxicity on MRC-5 cells (IC_{50} 7.5 μM) (Table 3). No activity at all was found against *C. albicans*, *E. coli* and *P. aeruginosa* (IC_{50} >200 μg/mL).

Table 3. Antiprotozoal activity of *G. mangostana* extracts and α-mangostin.

Sample	*P. falciparum*		*L. infantum*		*T. cruzi*		*T. brucei*		MRC-5
	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}
Dichloromethane extract	2.7 μg	3.5	7.5 μg	3.5	7.6 μg	1.2	0.5 μg	18.8	9.4 μg
Ethyl acetate extract	40.3 μg	>1.6	>64 μg	1	34.6 μg	1.9	56.4 μg	1.1	>64 μg
α-Mangostin	2.2 μM	3.4	8 μM	<1	8.9 μM	<1	7.9 μM	<1	7.5 μM

Table 4. Antimicrobial activity (IC_{50}) of α-mangostin.

	B. subtilis	*C. albicans*	*E. coli*	*P. aeruginosa*	*S. aureus*	*Mycobacterium*			
						smegmatis	*cheleneoi*	*xenopi*	*intracellulare*
MIC (μM)	3.9	>200			7.8	3.7	3.7	3.7	3.7

To the best of our knowledge, no data exist in the literature regarding the antiprotozoal activity and potential significance of *G. mangostana* as a source of antitrypanosomal and antiplasmodial compounds. *G. parvifolia* (Miq) has been used as a herbal remedy to treat malaria [21] and α-mangostin was found active against *P. falciparum* with IC_{50} values of 5.1 and 17 μM [22,23]. In our study, α-mangostin was found slightly more potent (IC_{50} 2.2 μM), but also cytotoxic to MRC-5 cells (IC_{50} 7.5 μM), hence suggesting a non-specific inhibition. The latter also explains the observed activity against *L. infantum*, *T. brucei* and *T. cruzi*, with IC_{50} values between 8.0 and 9.0 μM (Table 3). Another illustration of non-selectivity are several studies quoting the antimicrobial potential of *G. mangostana* extract [24,25]. However, the observed IC_{50} values may still justify the claimed (topical) uses of *G. mangostana* to treat infections in the traditional medicine.

This study clearly illustrates that interpretation of the antiprotozoal and antimicrobial potential of prenylated xanthones proves to be far from easy in view of the low level of selectivity. Available data in literature must be interpreted with great caution, particularly when parallel cytotoxicity data are not available. One route of further research on xanthones could be through structural modification with the sole option to maximize efficacy and reduce toxicity, e.g., non-selectivity.

3. Experimental Section

3.1. General

The UV and IR spectra were recorded on Hitachi-UV-3200 and JASCO 320-A spectrometers. The ^{1}H-, ^{13}C-NMR and 2D-NMR spectra were recorded on a Bruker AMX-500 spectrometer with tetramethylsilane (TMS) as internal standard. Chemical shifts are given in ppm (δ) relative to

tetramethylsilane internal standard and scalar coupling constants (*J*) are reported in Hertz. FAB and HRFABMS (neg. ion mode, matrix: glycerol) were registered on a JEOL JMS-HX110 mass spectrometer. Thin layer chromatography (TLC) was performed on precoated silica gel F254 plates (E. Merck, Darmstadt, Germany); detection was done at 254 nm and by spraying with *p*-anisaldehyde/H_2SO_4 reagent. All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

3.2. Plant Material

The fruits of *G. mangostana* Linn. were purchased from a local market at Riyadh city in 2009.

3.3. Extraction and Isolation

The air-dried pericarp (500 gm) was extracted by maceration with 70% ethanol (3 × 2 L) at room temperature. After filtration and evaporation of the solvent under vacuum, the combined ethanolic extract (70 gm) was suspended in water (200 mL) and successively partitioned with *n*-hexane (3 × 400 mL), dichloromethane (3 × 400 mL) and ethyl acetate (3 × 400 mL) to deliver the corresponding extracts. Based on pattern of separation and close similarity of compounds on TLC examination for both *n*-hexane and dichloromethane extracts, they were pooled together. The combined fractions were further purified by application onto the top of a silica gel packed column (Merck), eluted with *n*-hexane/ethyl acetate, followed by ethyl acetate/methanol solvent system gradient to give five fractions A–F. Fractions A, B and C were separately purified by chromatotron (Harrison Research, Palo Alto, California, CA, USA) using 5%, 15% and 20% ethyl acetate/*n*-hexane to give compound **3** (12 mg), **4** (8 mg) and **5** (20 mg). Direct crystallization of fractions D and E eluted by 30% and 40% ethyl acetate/*n*-hexane gave compound **2** (10 mg) and **1** (300 mg), while direct crystallization of fraction F eluted by 5% methanol/ethyl acetate gave compound **6** (20 mg).

3.4. Spectral Data (Tables 1 and 2)

Trihydroxy-7-methoxy-2,8-diprenylxanthone (α-mangostin) (**1**). Yellow amorphous powder; m.p. 180–182 °C; HREIMS: *m/z* = 410.1729 (calc. for $C_{24}H_{26}O_6$, 410.46). ^{1}H-NMR (DMSO, 500 MHz); ^{13}C-NMR (*d*-DMSO, 125 MHz): see Tables 1 and 2.

1,6-Dihydroxy-3,7-dimethoxy-2,8-diprenylxanthone-(β-mangostin) (**2**). Pale yellow crystal; m.p. 162–163 °C; HREIMS: *m/z* = 424 calc. for $C_{25}H_{28}O_6$, 424.46. ^{1}H-NMR (CDCl3, 500 MHz); ^{13}C-NMR ($CDCl_3$, 125 MHz): see Tables 1 and 2.

1-Hydroxy-3,6,7-trimethoxy-2,8-bis(3-methylbut-2-enyl) xanthone (**3**). Pale yellow gum; m.p. 152–154 °C; HREIMS: *m/z* = 438.5128 (calc. for $C_{26}H_{30}O_7$, 438.5128). ^{1}H NMR (CDCl3, 500 MHz); ^{13}C-NMR ($CDCl_3$, 125 MHz): see Tables 1 and 2.

9-Hydroxycalabaxanthone (**4**). Bright yellow needles; m.p. 152–154 °C; HREIMS: *m/z* = 408.1572 (calc. for $C_{24}H_{24}O_6$, 408.45). ^{1}H-NMR ($CDCl_3$, 500 MHz); ^{13}C-NMR ($CDCl_3$, 125 MHz): see Tables 1 and 2.

1,3,6-Trihydroxy-2,5-diprenyl-6',6'-dimethylpyrano(2',3':7,8) xanthone (tovophyllin A) (**5**). Yellow needles; m.p. 218–220 °C; HREIMS: *m/z* = 462.541 (calc. for $C_{28}H_{30}O_6$, 462.2042). ^{1}H-NMR ($CDCl_3$, 500 MHz); ^{13}C-NMR ($CDCl_3$, 125 MHz): see Tables 1 and 2.

3,5,7,3',4'-Pentahydroxyflavan (Catechin) (**6**). Cryst.; m.p. 175–177 °C; HREIMS: *m/z* = 290.0790 (calc. for $C_{15}H_{14}O_6$, 290.272). ^{1}H-NMR ($CDCl_3$, 500 MHz): 2.74 (*dd*, *J* = 16.5, 4.5 Hz, H-4a) 2.86 (*dd*, *J* = 16.5, 4.5 Hz, H-4b), 4.19 (*m*, H-3), 4.83 (br*s*, H-2), 5.94 (*d*, *J* = 1.5 Hz, H-6), 5.99 (*d*, *J* = 1.5 Hz, H-8), 6.78 (*dd*, *J* = 8.0, 1.0 Hz, H-6'), 6.81 (*d*, *J* = 8.0 Hz, H-5'),7.00 (*d*, *J* = 1.5 Hz, H-2'); ^{13}C-NMR (CD_3OD, 125 MHz): 80.2 (C-2), 67.5 (C-3), 29.5 (C-4), 157.7 (C-5), 96.5 (C-6), 158.0 (C-7), 96.0 (C-8), 157.4 (C-9), 100.2 (C-10), 132.3 (C-1'), 115.4 (C-2'), 146.0 (C-3'), 145.8 (C-4'), 116.0 (C-5'), 119.5 (C-6').

3.5. Reference Drugs

For the different tests, appropriate reference drugs were used as positive control: tamoxifen for MRC-5, chloroquine for *P. falciparum*, miltefosine for *L. infantum*, benznidazole for *T. cruzi* and suramin for *T. brucei*. All reference drugs were either obtained from the fine chemical supplier Sigma-Aldrich (Taufkirchen, Germany; tamoxifen, suramin) or from WHO-TDR (Geneva, Switzerland; chloroquine, miltefosine, benznidazole).

3.6. Biological Assays

The integrated panel of microbial screens and standard screening methodologies were adopted as previously described [26]. All assays were performed in triplicate at the Laboratory of Microbiology, Parasitology and Hygiene at the University of Antwerp (Antwerp, Belgium). Extracts were tested at five concentrations (64, 16, 4, 1 and 0.25 μg/mL) to establish a full dose-titration and determination of the IC_{50} (inhibitory concentration 50%). The final in-test concentration of DMSO did not exceed 0.5%, which is known not to interfere with the different assays [26]. The selectivity of activity was assessed by simultaneous cytotoxicity evaluation on the MRC-5 fibroblast cell line. The criterion for activity was an IC_{50} <10 μg/mL and a selectivity index (SI) of >4.

3.6.1. Antiplasmodial Activity

Chloroquine-resistant *P. falciparum* K 1-strain was cultured in human erythrocytes O^+ at 37 °C under a low oxygen atmosphere (3% O_2, 4% CO_2, and 93% N_2) in RPMI-1640, supplemented with 10% human serum. Infected human red blood cells (200 μL, 1% parasitaemia, 2% haematocrit) were added to each well and incubated for 72 h. After incubation, test plates were frozen at −20 °C. Parasite multiplication was measured using the Malstat assay, a colorimetric method based on the reduction of 3-acetyl pyridine adenine dinucleotide (APAD) by parasite-specific lactate-dehydrogenase (pLDH) [26,27].

3.6.2. Antileishmanial Activity

L. infantum MHOM/MA (BE)/67 amastigotes were collected from the spleen of an infected donor hamster and used to infect primary peritoneal mouse macrophages. To determine *in vitro*

antileishmanial activity, 3×10^4 macrophages were seeded in each well of a 96-well plate. After 2 days outgrowth, 5×10^5 amastigotes/well, were added and incubated for 2 h at 37 °C. Pre-diluted plant extracts were subsequently added and the plates were further incubated for 5 days at 37 °C and 5% CO_2. Parasite burdens (mean number of amastigotes/macrophage) were microscopically assessed on 500 cells after Giemsa staining of the testplates, and expressed as a percentage of the blank controls without plant extract.

3.6.3. Antitrypanosomal Activity

Trypanosoma brucei Squib-427 strain (suramin-sensitive) was cultured at 37 °C and 5% CO_2 in Hirumi-9 medium, supplemented with 10% fetal calf serum (FCS) [28]. About 1.5×10^4 trypomastigotes/well were added to each well and parasite growth was assessed after 72 h at 37 °C by adding resazurin [29]. For Chagas disease, *T. cruzi* Tulahuen CL2 (benznidazole-sensitive, LacZ-reporter strain) [30] was maintained on MRC-5 cells in minimal essential medium (MEM) supplemented with 20 mM L-glutamine, 16.5 mM sodium hydrogen carbonate and 5% FCS. In the assay, 4×10^3 MRC-5 cells and 4×10^4 parasites were added to each well. After incubation at 37 °C for 7 days, parasite growth was assessed by adding the substrate chlorophenol red α-D-galactopyranoside. The color reaction was read at 540 nm after 4 h and absorbance values were expressed as a percentage of the blank controls.

3.6.4. Antimicrobial Activity

Samples were tested for antimicrobial activity according to the Clinical Laboratory Standard Institution using American type of Culture Collection (ATCC) standard [31].

3.6.5. Cytotoxicity Assay

MRC-5 SV_2 cells were cultivated in MEM, supplemented with L-glutamine (20 mM), 16.5 mM sodium hydrogen carbonate and 5% FCS. For the assay, 10^4 MRC-5 cells/well were seeded onto the test plates containing the pre-diluted sample and incubated at 37 °C and 5% CO_2 for 72 h. Cell viability was assessed fluorimetrically after 4 h of addition of resazurin. Fluorescence was measured (excitation 550 nm, emission 590 nm) and the results were expressed as % reduction in cell viability compared to control [26].

4. Conclusions

Interpretation of the antiprotozoal and antimicrobial potential of prenylated xanthones proves to non-conclusive in view of the low level of selectivity. One route of further research on this subject could be through structural modification with the sole option to maximize efficacy and avoid non-selectivity.

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Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds **1**–**6** are available from the authors.