View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

South African Journal of Botany 73 (2007) 347–354

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

Antimicrobial components of the methanolic extract from the stem bark of *Garcinia smeathmannii* Oliver (Clusiaceae)

V. Kuete [a,*], J. Komguem [b], V. Penlap Beng [a], A.L. Meli [b], J.G. Tangmouo [b], F.-X. Etoa [a], D. Lontsi [b]

[a] *Department of Biochemistry, Faculty of Science, University of Yaoundé I, P.O. Box. 812 Yaoundé, Cameroon*

[b] *Department of Organic Chemistry, University of Yaoundé I, P.O. Box. 812 Yaoundé, Cameroon*

Received 7 October 2006; received in revised form 30 November 2006; accepted 25 January 2007

Abstract

The methanolic extract (GSM) prepared from the stem bark of *Garcinia smeathmannii* as well as ten compounds isolated from this crude extract, were tested for their antimicrobial activity against Gram-positive bacteria (6 species), Gram-negative bacteria (12 species) and 3 *Candida* species using well micro-dilution methods. The GSM showed very interesting inhibition effects on the growth of the tested pathogens with the minimal inhibition concentrations (MIC) lower than 156.25 µg/mL on 21 of the 22 pathogens tested. Purified compounds showed selective activities. Two of these compounds namely Cheffouxanthone (**1**) and Friedelin (**9**) exhibited both antibacterial and anticandidal activities. The antimicrobial activity of compounds **1**, Bangangxanthone A (**4**), and Guttiferone I (**7**), as well as that of GSM is being reported for the first time. The overall results provide promising baseline information for the potential use of the crude extract from the stem bark of *G. smeathmannii* as well as some of the isolated compounds in the treatment of bacterial and fungal infections.

© 2007 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: *Garcinia smeathmannii*; Compounds; Antimicrobial activity

1. Introduction

Infectious diseases constitute one of the main problems that modern medicine had to face these last 30 years. Despite the high proportion of efficient antibiotics available nowadays, the emergence of resistant microorganisms has lowered their potency (Bacq-Calberg et al., 1999). These problems, along with the high incidence of poverty within population orientated the African population more and more towards the folk medicine. Today, about 80% of Africans ask for help to tradi-therapists or herbalists in the treatment of various diseases. In Cameroon, many medicinal plants from Clusiaceae family are used as herbal medicines. Within this family, plants of *Garcinia* genus, which grow in the lowland tropical rain forest of West Africa and Asia have been reported to possess antimicrobial properties (Watt and Breyer-Brandwijk, 1962; Hiroyuki et al., 1996). Xanthones and flavonoids were found to be the major compounds associated to the therapeutic potential of *Garcinia* species (Hiroyuki et al., 1996; Waterman and Hussain, 1982; Iwu et al., 1990). Among the different species of *Garcinia* genus, *Garcinia smeathmannii* Oliver is extensively used by the local population of Cameroon for the treatment of bacterial and fungal infections. The antimicrobial activity of some of the compounds from the stem bark of this plant such as Smeathxanthones A and B, was reported in our previous paper (Komguem et al., 2005). Nevertheless, the inhibitory activity of the crude extract of this plant is not yet documented to the best of our knowledge. This study therefore reports the antimicrobial activity of this extract, with that of a number of compounds isolated from this extract.

2. Materials and methods

2.1. General experimental procedures

Melting points were recorded on a Cipla I-28 digital apparatus and were uncorrected. Aluminium sheet pre-coated with silica gel 60 $F_{254\ nm}$ (Mereck) was used for thin layer chromatography and the isolated spots were visualized using both ultra-violet light (254 and 366 nm) and 10% H_2SO_4 spray reagent. The chemical structure of each of the isolated

* Corresponding author. Tel.: +237 735 59 27/+237 533 84 55; fax: +237 222 60 18.

E-mail address: kuetevictor@yahoo.fr (V. Kuete).

0254-6299/$ - see front matter © 2007 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2007.01.004

compound was determined on the basis of spectroscopic data produced by one and two-dimensional nuclear magnetic resonances (NMR), recorded on Brüker DRX-400 instrument. This spectrometer was equipped with 5-mm, ^{1}H- and ^{13}C-NMR probes operating at 400 and 100 MHz, with tretramethylsilane as internal standard. Mass spectra were recorded on a API QSTAR pulsar mass spectrometer. The structures of the compounds (Figs. 1 and 2) were confirmed by their spectroscopic data in accordance with that of reference from available literature.

2.2. Plant material

The stem bark of *G. smeathmannii* was collected in Baham, in the West province of Cameroon. A botanist, Mr. Victor Nana in the national herbarium of Cameroon, identified the plant. The voucher specimen was deposited under the reference number 35169/HNC.

2.3. Extraction

The plant material was finely cut into pieces, dried and ground to yield a powder. Then, 5 kg of this powder were macerated in methanol (20 L) for 72 h. The filtrate obtained using Whatman filter paper no. 1 was concentrated under vacuum to give crude extract (180 g; 3.6%). This was stored at 4 °C till further use.

2.4. Isolation and identification of compounds

One hundred grams (100 g) of crude extract were exhausted with hexane to give 18 g of hexanic fraction (A) and 73 g of remaining extract (B). Fraction (A) was subjected to silica gel 60 (0.063–0.200 mm) column chromatography, using hexane–EtOAc gradient (99:1 to 85:15) as eluent at 2 mL min. Sub-fractions of 100 mL each were collected, concentrated under vacuum and pooled according to TLC analysis. This separation yielded Friedelin $C_{30}H_{50}O$ (**9**) (White crystals; 30 mg in hexane–EtOAc acetate 95:5; m.p.: 265–266; *m/z* 426) (Gunatilaka et al., 1982), Smeathxanthone B $C_{23}H_{22}O_6$ (**5**) (yellow crystals; 120 mg in hexane–EtOAc 90:10; Rf: 0.27 using hexane–EtOAc 95:5; m.p.: 187–189; *m/z* 394) (Komguem et al., 2005) and one major sub-fraction (A1; 4 g obtained in hexane–Ethyl acetate 90:10 to 85:15). A1 was chromatographed over silica gel 60 using CH_2Cl_2–MeOH (98:2) elution system at 1 mL min. Sub-fractions of 15 mL each were collected and the separation yielded Triacontanyl cafeate $C_{39}H_{68}O_4$ (**10**) (75 to 285 mL; white crystals; 17 mg; m.p.: 103–104, *m/z* 600) (Hesham et al., 2003), and Guttiferone I $C_{43}H_{58}O_6$ (**7**) (yellow oil; 330 to 525 mL; 25 mg; *m/z* 670; Rf: 0.38 using CH_2Cl_2–MeOH 95:5) (Herath et al., 2005).

Fifty grams (50 g) of fraction B were subjected to flash chromatography over silica gel 60 using hexane–EtOAc gradient (95:5 to 75:25) at 2 mL min. The sub-fractions of 150 mL each were collected, concentrated under vacuum and pooled on the basis of analytic TLC. This afforded 2 major sub-fractions namely B1 (hexane–EtOAc 90:10; 6.15 L, 12 g) and B2 (hexane–EtOAc 85:15 to 80:20; 5.25 L, 16 g). Further silica gel 60 column chromatography of B1 using hexane–EtOAc gradient (90:10 to 80:20) at 1 mL min yielded Cheffouxanthone $C_{23}H_{24}O_6$ (**1**) (yellow crystals; 25 mg in hexane–EtOAc 90:10; m.p.: 155–158; *m/z* 396; Rf: 0.33 using hexane–EtOAc 95:5;) (Meli et al., 2006), Smeathxanthone A $C_{23}H_{24}O_6$ (**6**) (yellow crystals; 65 mg in hexane–EtOAc 88:12; m.p.: 216–218; *m/z* 396; Rf: 0.67 using hexane–EtOAc 95:5) (Komguem et al., 2005), and 1,5 dihydroxyxanthone $C_{13}H_8O_4$ (**2**) (yellow crystals; 35 mg in hexane–EtOAc 85:15; m.p.: 264–266; *m/z*: 228) (Gunasekera, 1975).

The purification of B2 over silica gel 60 using hexane–EtOAc gradient (85:15 to 75:25) at 1 mL min yielded 1,3,5-trihydroxyxanthone $C_{13}H_8O_5$ (**3**) (yellow crystals; 20 mg in hexane–EtOAc 85:15; m.p.: 302–305; *m/z* 244) (Locksley and Murray, 1971), Isoxanthochymol $C_{38}H_{50}O_6$ (**8**) [yellow crystals; 23 mg in hexane–EtOAc 80:20; $[\alpha]_D$ + 186 (c 0.06, CH_3COCH_3); m.p.: 246–248; *m/z* 602] (Iinuma et al., 1996) and another 7 g of sub-fraction (B1.1) essentially obtained with the system hexane–EtOAc 90:10. B1.1 was finally column chromatographed over silica gel 60 using petroleum ether–

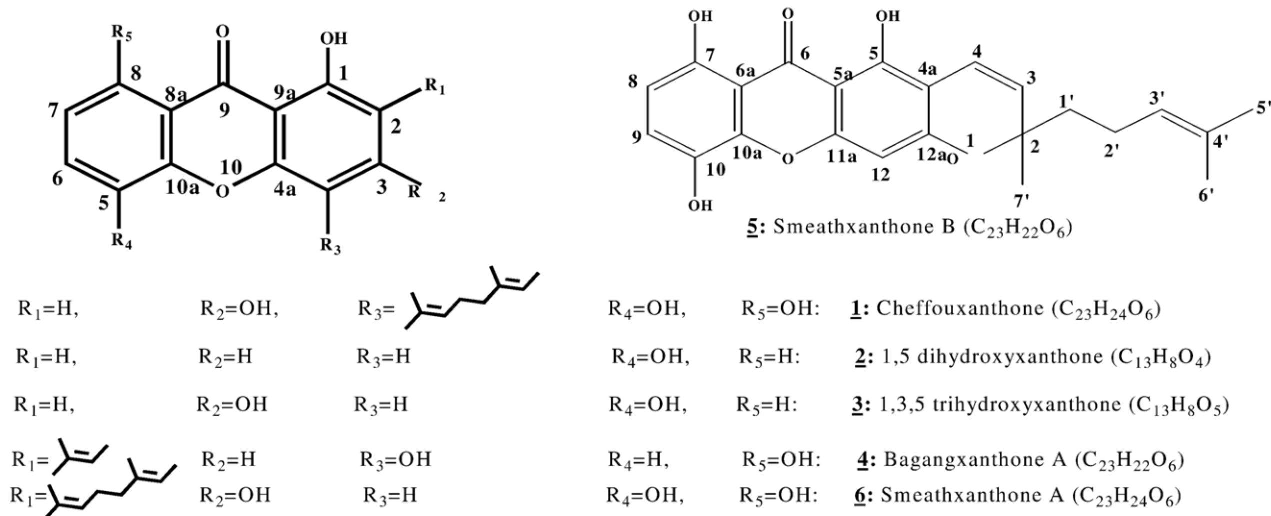

Fig. 1. Chemical structures of xanthones isolated from the stem bark of *Garcinia smeathmannii.*

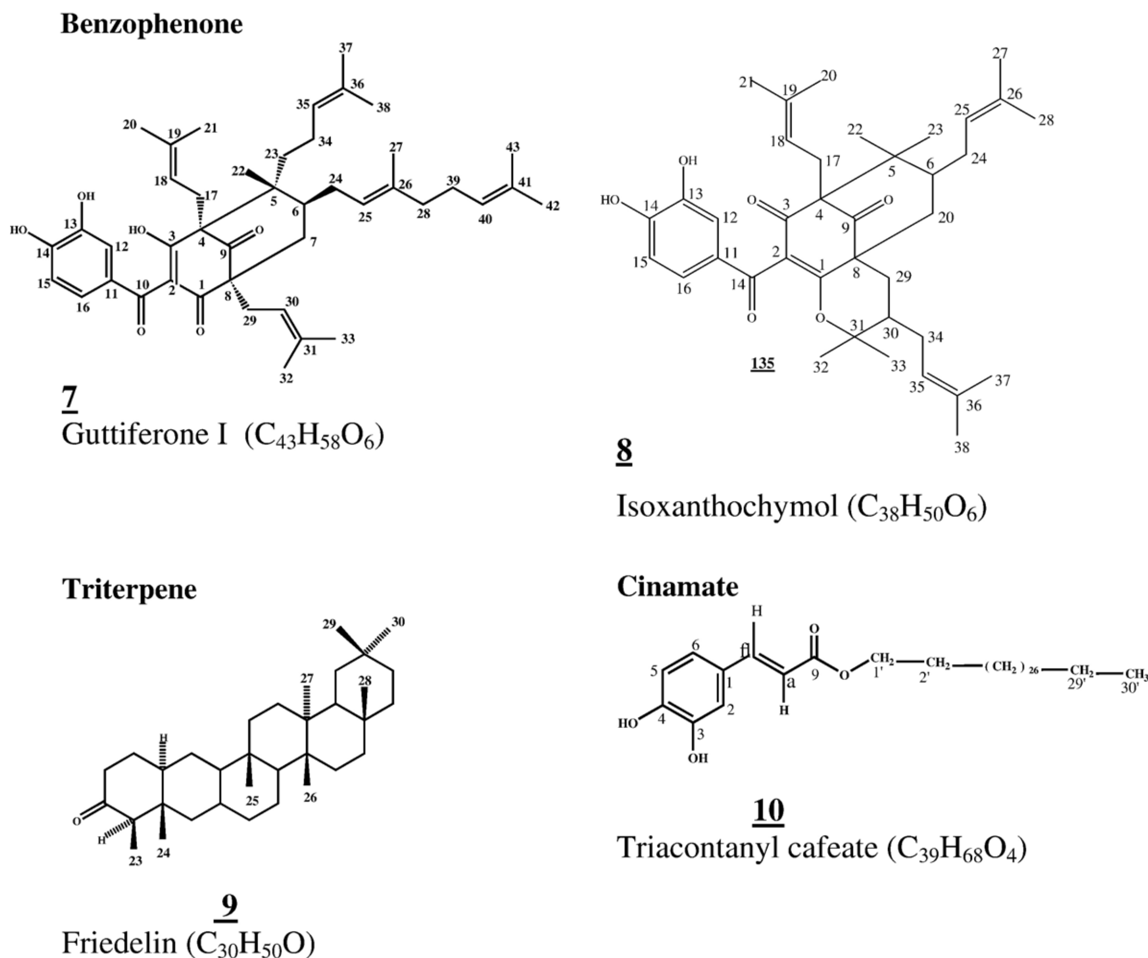

Fig. 2. Chemical structures of the Benzophenones, triterpene and cinamate isolated from the stem bark of *Garcinia smeathmannii*.

EtOAc 85:5 system for elution at 1 mL min. the sub-fractions of 20 mL each were collected. This purification yielded Bangangxanthone A $C_{23}H_{22}O_6$ (**4**) (yellow crystals; 140 to 660 mL; 45 mg; m.p.: 199–201; *m/z* 394; Rf: 0.38 using hexane–EtOAc 95:5) (Meli et al., 2005).

2.5. Preliminary phytochemical analysis of the crude extract

The major groups of secondary metabolites of GSM were screened using the common methods described by Harbone (1973).

2.6. Preparation of the standard inoculum

The cell suspension of about 1.5 ċ 10^6 CFU/mL obtained from a McFarland turbidity standard no. 0.5 was used in the antimicrobial testing. The suspension was standardised by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120–01 spectrophotometer) (Tereschuk et al., 1997).

2.7. Antimicrobial assays

The MICs of the tested samples and the reference antibiotics were determined as follows: the tested sample was first of all dissolved in DMSO and the solution obtained was added to the phenol red (0.01%) containing-nutrient broth, and supplemented with 10% glucose (NBGP) to make a final concentration of 156.25 µg/mL for crude extract and 19.53 µg/mL for the isolated compounds or the reference antibiotics. This was serially diluted two fold to obtain concentration ranges of 0.61 to 156.25 µg/mL and 0.038 to 19.53 µg/mL for crude extract and purified compounds respectively. One hundred microliter (100 µL) of each concentration was added in a well (96-well microplate) containing 95 µL of NBGP and 5 µL of standard inoculum. The final concentration of DMSO in the well was less than 1% (preliminary analyses with 1% (v/v) DMSO/NBGP affected neither the growth of the test organisms nor the change of colour due to this growth). The negative control wells, consisted of 195 µL of NBGP and 5 µL of the standard inoculum (Zgoda and Porter, 2001). The plates were covered with a sterile plate sealer, then agitated to mix the content of the wells using a plate shaker, then incubated at 37 °C for 24 h. Each assay was repeated twice. The microbial growth was determined by observing the change of colour in the wells (red when there is no growth and yellow when there is growth). The lowest concentration showing no colour change was considered as the MIC.

For the determination of MMC, a portion of liquid (5 µL) from each well that showed no change in colour was plated on

the Mueller Hinton Agar and incubated at 37 °C for 24 h. The lowest concentration that yielded no growth (no colony of microorganism on the Mueller Hinton Agar medium) after this sub-culturing was taken as the MMC (Carbonnelle et al., 1987).

3. Results and discussion

The preliminary phytochemical analysis of GSM indicated the presence of the group compounds such as alkaloids, phenols, polyphenols, saponins, tannins, triterpenes, anthraquinones, flavonoids and steroids. Many compounds belonging to these secondary metabolite groups have been reported to their antimicrobial activities (Cowan, 1999).

The chemical structures of the isolated compounds (Figs. 1 and 2) were elucidated by their ^{1}H-NMR spectroscopic. They were identified as xanthones [Cheffouxanthone (**1**); 1,5 dihydroxyxanthone (**2**); 1,3,5-trihydroxyxanthone (**3**); Bangangxanthone A (**4**); Smeathxanthone B (**5**); Smeathxanthone A (**6**)], benzophenone [Guttiferone I (**7**); Isoxanthochymol (**8**)], triterpene [Friedelin (**9**)] and cinamate [Triacontanyl cafeate (**10**)]. This result is in accordance with the preliminary phytochemical analysis, since all of the isolated compounds belong to the investigated group of secondary metabolites. Our research group has previously reported the isolation of compounds **2**, **4** and **9** from *Garcinia polyanta* (Meli et al., 2005), compounds **1** (Meli et al., 2006), **3**, **5**, and **6** from *G. smeathmannii* (Komguem et al., 2005). Also, compound **7** has been isolated recently from *Garcinia humulis* (Herath et al., 2005).

The methanolic extract showed a very interesting inhibition effects on the growth of the tested microorganisms with MIC < 156.25 µg/mL on 21 of the 22 tested microbial species (Table 1). Only *Salmonella typhimurium* was found to be resistant to this extract. However, the growth inhibition of *S. typhimurium* could also be expected at MIC > 156.25 µg/mL, as compounds **9** and **10** were found to possess inhibitory effects on the pathogen. The presence of the antimicrobial components from the investigated metabolite groups could eventually explain the inhibitory potency of the methanolic extract of this plant. The observed activity could also explain the traditional use of this plant in the treatment of infectious diseases.

Purified compounds showed selective activities. Two of the ten isolated compounds (**1** and **9**) exhibited both antibacterial and anticandidal activities at 19.53 µg/mL limit MIC value tested (Table 1). Considering the limit MIC value retained in this work (19.53 µg/mL), compounds **1** and **9** presented the most important antimicrobial spectra; their inhibitory activities have been observed respectively on 13 and 12 of the 22 tested microorganisms. The MIC values varied from 0.6 µg/mL (1.02 µM) to 4.88 µg/mL (8.10 µM) for compound **9** while the corresponding interval ranged from 4.88 µg/mL (12.33 µM) to 19.53 µg/mL (49.3 µM) for compound **1** (Table 1). Other compounds showed microbial growth inhibition on nine (**4**), eight (**3** and **10**), seven (**2**), five (**7**) and two (**5**, **6** and **8**) of the 22 tested microorganisms. Though, the tested compounds presented spectra lower than that of the reference antibiotics, the degree of sensitivity of the inhibited microorganisms can be considered as very interesting. However, the MIC values obtained with compounds **3**, **4**, **7** and **9** were lower than that of the reference antibiotics on all inhibited bacteria and yeasts. Furthermore, other compounds have presented the MIC values lower than that of the reference antibiotics on at least one of the tested microorganisms. The lowest MIC value of 0.076 µg/mL or 0.16 µM for compound **4** on *Pseudomonas aeruginosa* is 5.62 times lower than that of Gentamycin (0.9 µM). Apart from the MIC values of **1** on *Bacillus stearothermophilus* and *Candida gabrata*, that of **10** on *S. typhimurium*, all tested compounds gave values lower than 19.53 µg/mL on the sensitive microorganisms. The results from the Minimal Microbicidal Concentration (MMC) determination (Table 2) showed that the values lower than 156.25 µ/mL were obtained with GSM on 14 of the 21 sensitive microbial species. A keen look of the results of Tables 1 and 2, the MIC values are 4 times lower than the MMCs on corresponding (sensitive) microorganisms showing that the microbicidal effects could be expected (Carbonnelle et al., 1987).

Numerous studies have documented the antimicrobial potency of the crude extracts from genus *Garcinia* as well as that of some of their antimicrobial components. Mackeen et al. (2000) reported the antibacterial and antifungal activities of crude methanolic extract from different parts of *Garcinia atroviridis*. Significant antifungal activity of *G. atroviridis* against *Cladosporium herbarum* was most notably noted with the fruit, and the leaf extracts (Mackeen et al., 2000). The antimicrobial principles of this genus were generally found to be xanthones such as cowaxanthones from *Garcinia cowa* (Panthong et al., 2006), parvifolixanthones from *Garcinia parvifolia* (Rukachaisirikul et al., 2006), dulcisxanthones from *Garcinia dulcis* (Deachathai et al., 2006), tetraprenylated xanthones, named scortechinones from *Garcinia scortechinii* (Rukachaisirikul et al., 2000), and phloroglucinols from *G. parvifolia* (Rukachaisirikul et al., 2006). Also α-mangostin, isolated from the stem bark of *Garcinia mangostana* L., was found to be active against vancomycin resistant enterococci and methicillin resistant *Staphylococcus aureus* (Sakagami et al., 2005). The inhibitory potency of xanthones isolated from *G. smeathmannii,* corroborate therefore with their findings. Though the antimicrobial activity of triterpenes from *Garcinia* species is not well documented, Friedelin is a well-known antibiotic (Kuete et al., 2007).

The results presented in the present paper can be considered as very promising in the perspective of new drugs discovery from plant sources. The antimicrobial activity of crude extract from *G. smeathmannii* as well as that of Cheffouxanthone (**1**), Bangangxanthone A (**4**) and Guttiferone I (**7**) is being reported for the first time. However, the antimicrobial potencies of compounds **5** and **6** on very limited number of strains were documented in our previous paper (Komguem et al., 2005). Though presenting very weak antimicrobial spectra in the present study, it has been shown that compounds **5** and **6** are potential antimicrobials at higher doses (Komguem et al., 2005). Kilham (2004) has also demonstrated good antibacterial activity (mostly against *S. aureus*), a good antifungal activity against *Pseudallescheria boydii*, and a moderate activity against *Trichophyton schoenleinii* of compound **9**. The inhibition potency of many benzophenones, including compound **8** has also been

Table 1
Minimal inhibition concentration (MIC) of compounds from the stem bark of *Garcinia smeathmannii* and reference antibiotics

Microbial strains [a]	MIC in µg/mL and in µM (in parenthesis) of tested samples [b]											
	GSM	1	2	3	4	5	6	7	8	9	10	RA [c]
Gram-negative bacteria												
C. freundii	156.25	9.76 (24.65)	–	1.22 (6.35)	0.61 (1.26)	–	–	1.22 (1.96)	–	–	9.76 (22.93)	4.88 (0.9)
E. aerogens	78.12	–	–	–	–	–	–	–	–	–	–	9.76 (1.8)
E. cloaclae	156.25	9.76 (24.65)	–	–	0.61 (1.26)	–	–	1.22 (1.96)	–	0.61 (1.02)	–	4.88 (0.9)
E. coli	39.06	–	–	–	0.61 (1.26)	–	–	–	–		–	1.22 (0.23)
K. pneumonia	78.12	–	–	0.31 (1.59)	–	–	–	–	–	–	–	2.44 (0.45)
M. morganii	78.12	9.76 (24.65)	9.76 (55.45)	–	0.15 (0.31)	–	–	–	–	–	9.76 (22.93)	2.44 (0.45)
P. mirabilis	156.25	–	–	–	–	–	–	–	–	–	–	2.44 (0.45)
P. vulgaris	78.12	–	–	1.22 (6.35)	–	–	–	1.22 (1.96)	–	1.22 (2.03)	–	1.22 (0.23)
P. aeruginosa	78.12	–	–	–	0.076(0.16)	–	–	–	–	–	0.61 (1.43)	4.88 (0.9)
S. dysenteria	156.25	9.76 (24.65)	9.76 (55.45)	0.61 (3.18)	0.61 (1.26)	–	–	–	–	1.22 (2.03)	–	2.44 (0.45)
S. flexneri	78.12	9.76 (24.65)	9.76 (55.45)	0.61 (3.18)	1.22 (2.51)	–	–	–	–	1.22 (2.03)	–	2.44 (0.45)
S. typhi	78.12	–	–	–	–	–	–	–	–	0.61 (1.02)	9.76 (22.93)	2.44 (0.45)
S. typhimurium	–	–	–	–				–	–	1.22 (2.03)	19.53 (45.85)	1.22 (0.23)
Gram-positive bacteria												
B. cereus	156.25	9.76 (24.65)	4.88 (27.73)	1.22 (6.35)		4.88 (12.29)	9.76 (24.77)	–	9.76 (16.21)	–	4.88 (11.46)	1.22 (0.23)
B. megaterium	78.12	9.76 (24.65)	1.22 (6.93)	1.22 (6.35)	–	–	–	0.61 (0.98)	–	1.22 (2.03)	2.44 (5.73)	2.44 (0.45)
B. stearothermophilus	156.25	19.53 (49.3)	–	–	0.61 (1.26)	–	–	–	4.88 (8.10)	1.22 (2.03)	–	4.88 (0.9)
B. subtilis	39.06	–	2.44 (13.86)	–	–	9.76 (24.58)	9.76 (24.77)	–	–	–	–	1.22 (0.23)
S. faecalis	78.12	9.76 (24.65)	0.61 (3.46)	0.61 (3.18)	0.61 (1.26)	–	–	0.61 (0.98)	–	0.61 (1.02)	2.44 (5.73)	2.44 (0.45)
S. aureus	156.25	9.76 (24.65)	–	–	–	–	–	–	–	–	–	1.22 (0.23)
Yeasts												
C. albicans	156.25	9.76 (24.65)	–	–	–	–	–	–	–	2.44 (4.05)	–	9.76 (1.04)
C. krusei	156.25	4.88 (12.33)	–	–	–	–	–	–	–	4.88 (8.10)	–	4.88 (0.52)
C. gabrata	156.25	19.53 (49.3)	–	–	–	–	–	–	–	2.44 (4.05)	–	9.76 (1.04)

[a] Microbial strains: *C. freundii*: *Citrobacter freundii*; *E. aerogens*: *Enterobacter aerogens*; *E. cloaclae*: *Enterobacter cloaclae*; *E. coli*: *Escherichia coli*; *K. pneumonia*: *Klebsiella pneumonia*; *M. morganii*: *Morganella morganii*; *P. mirabilis*: *Proteus mirabilis*; *P. vulgaris*: *Proteus vulgaris*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. dysenteria*: *Shigella dysenteria*; *S. flexneri*: *Shigella flexneri*; *S. typhi*: *Salmonella typhi*; *S. typhimurium*: *Salmonella typhimurium*; *B. cereus*: *Bacillus cereus*; *B. stearothermophilus*: *Bacillus stearothermophilus*; *B. subtilis*: *Bacillus subtilis*; *S. faecalis*: *Streptococcus faecalis*; *S. aureus*: *Staphylococcus aureus*; *C. albicans*: *Candida albicans*; *C. krusei*: *Candida krusei*; *Candida gabrata*: *Candida gabrata*.

[b] Tested samples [GSM: methanolic extract, Cheffouxanthone (**1**); 1,5 dihydroxyxanthone (**2**); 1,3,5-trihydroxyxanthone (**3**); Bangangxanthone A (**4**); Smeathxanthone B (**5**); Smeathxanthone A (**6**), Guttiferone I (**7**); Isoxanthochymol (**8**), Friedelin (**9**) and cinamate Triacontanyl cafeate (**10**)].

[c] RA: reference antibiotics (Gentamycin for bacteria, Nystatin for yeast); (–): MIC >156.25 and 19.53 µg/mL respectively for GSM and pure compounds.

Table 2
Minimal microbicidal concentration (MMC) of compounds from the stem bark of *Garcinia smeathmannii* and reference antibiotics

Microbial strains[a]	MMC in μg/mL and in μM (in parenthesis) of tested samples[b]											
	GSM	1	2	3	4	5	6	7	8	9	10	RA[c]
Gram-negative bacteria												
C. freundii	>156.25	19.53 (49.3)	–	9.76 (25.4)	1.22 (2.51)	–	–	2.44 (3.92)	–	–	19.53 (45.85)	9.76 (1.8)
E. aerogens	156.25	–	–	–	–	–	–	–	–	–	–	19.53 (3.6)
E. cloaclae	>156.25	9.76 (24.65)	–	–	1.22 (2.51)	–	–	2.44 (3.92)	–	1.22 (2.03)	–	9.76 (1.8)
E. coli	78.12	–	–	–	1.22 (2.51)	–	–	–	–		–	2.44 (0.45)
K. pneumonia	>156.25	–	–	0.61 (3.18)	–	–	–	–	–	–	–	4.88 (0.90)
M. morganii	156.25	19.53 (49.3)	19.53 (90.9)	–	0.61 (1.26)	–	–	–	–	–	19.53 (45.85)	4.88 (0.90)
P. mirabilis	>156.25	–	–	–	–	–	–	–	–	–	–	4.88 (0.90)
P. vulgaris	156.25	–	–	1.22 (6.35)	–	–	–	2.44 (3.92)	–	4.88 (8.12)	–	2.44 (0.45)
P. aeruginosa	156.25	–	–	–	0.15 (0.31)	–	–	–	–	–	1.22 (2.86)	9.76 (1.8)
S. dysenteria	156.25	9.76 (24.65)	19.53 (90.9)	1.22 (6.35)	1.22 (2.51)	–	–	–	–	2.44 (4.06)	–	4.88 (0.90)
S. flexneri	78.12	9.76 (24.65)	9.76 (55.45)	1.22 (6.35)	2.44 (5.02)	–	–	–	–	2.44 (4.06)	–	4.88 (0.90)
S. aureus	>156.25	19.53 (49.3)	–	–	–	–	–	–	–	–	–	2.44 (0.45)
S. typhi	>156.25	–	–	–	–	–	–	–	–	1.22 (2.03)	19.53 (45.85)	4.88 (0.90)
S. typhimurium	–	–	–	–	–	–	–	–	–	4.88 (8.12)	>19.53 (45.85)	2.44 (0.45)
Gram-positive bacteria												
B. cereus	156.25	9.76 (24.65)	9.76 (55.45)	2.44 (12.7)		9.76 (24.58)	19.53 (49.54)	–	19.53 (32.42)	–	9.76 (22.93)	2.44 (0.45)
B. megaterium	156.25	19.53 (49.3)	4.88 (27.73)	2.44 (12.7)	–	–	–	1.22 (1.96)	–	2.44 (4.06)	4.88 (11.43)	4.88 (0.90)
B. stearothermophilus	156.25	>19.53 (49.3)	–	–	1.22 (2.51)	–	–	–	9.76 (16.21)	4.88 (8.12)	–	9.76 (1.8)
B. subtilis	78.12	–	4.88 (27.73)	–	–	19.53 (49.16)	19.53 (49.54)	–	–	–	–	2.44 (0.45)
S. faecalis	78.12	9.76 (24.65)	1.22 (6.93)	1.22 (6.35)	1.22(2.51)	–	–	1.22 (1.96)	–	2.44 (4.06)	9.76 (22.93)	4.88 (0.90)
Yeasts												
C. albicans	>156.25	19.53 (49.3)	–	–	–	–	–	–	–	–	–	19.53 (2.08)
C. krusei	>156.25	9.76 (24.65)	–	–	–	–	–	–	–	–	–	9.76 (1.04)
C. gabrata	156.25	>19.53 (49.3)	–	–	–	–	–	–	–	–	–	19.53 (2.08)

[a] Microbial strains: *C. freundii*: *Citrobacter freundii*; *E. aerogens*: *Enterobacter aerogens*; *E. cloaclae*: *Enterobacter cloaclae*; *E. coli*: *Escherichia coli*; *K. pneumonia*: *Klebsiella pneumonia*; *M. morganii*: *Morganella morganii*; *P. mirabilis*: *Proteus mirabilis*; *P. vulgaris*: *Proteus vulgaris*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. dysenteria*: *Shigella dysenteria*; *S. flexneri*: *Shigella flexneri*; *S.typhi*: *Salmonella typhi*; *S. typhimurium*: *Salmonella typhimurium*; *B. cereus*: *Bacillus cereus*; *B. stearothermophilus*: *Bacillus stearothermophilus*; *B. subtilis*: *Bacillus subtilis*; *S. faecalis*: *Streptococcus faecalis*; *S. aureus*: *Staphylococcus aureus*; *C. albicans*: *Candida albicans*; *C. krusei*: *Candida krusei*; *Candida gabrata*: *Candida gabrata*.

[b] Tested samples [GSM: methanolic extract, Cheffouxanthone (**1**); 1,5 dihydroxyxanthone (**2**); 1,3,5-trihydroxyxanthone (**3**); Bangangxanthone A (**4**); Smeathxanthone B (**5**); Smeathxanthone A (**6**), Guttiferone I (**7**); Isoxanthochymol (**8**), Friedelin (**9**) and cinamate Triacontanyl cafeate (**10**)].

[c] RA: reference antibiotics (Gentamycin for bacteria, Nystatin for yeast); (–): not tested because MIC was not determined.

demonstrated against methycillin-resistant *S. aureus* (Iinuma et al., 1996). The tested compounds are potentially responsible for the antimicrobial activity of GSM. The results obtained with GSM and compound **4** on *Pseudomonas aeruginosa* is very interesting due to the fact that this microorganism has emerged as one of the most problematic Gram-negative pathogens, with alarmingly high antibiotics resistance rates (Bacq-Calberg et al., 1999; Gangoué, 2000; Savafi et al., 2005). Even with the most effective antibiotics against this pathogen, namely carbapenems (imipenem and meropenem), the resistance rates were detected as 15% to 20.4% among 152 *P. aeruginosa* strains (Savafi et al., 2005). Also, the results obtained for all the microorganisms tested were interesting, due to the fact that they were all selected as multiresistant strains and that they are medically very important. *Bacillus* species especially *B. cereus* is an agent of food poisoning (Avril, 1997; Sleigh and Timbury, 1998;). *S. typhimurium* is etiologically the most important agent of food toxi-infections (Avril, 2000). *Candida albicans* and other *Candida* species, causing candidiasis, are increasingly important diseases worldwide distributed, due to the fact that they are frequent opportunistic pathogen in AIDS patients (Cowan, 1999). The incidence of the typhoid fever caused by *Salmonella typhi* is increasing in developing country nowadays.

The antimicrobial mechanisms associated to each group of chemical to which the isolated compounds belong, may explain the inhibition potency of the tested samples.

Membrane disruption could be suggested as one of the likely mechanisms of action of compound **9**, a triterpene (Cowan, 1999; Arvind et al., 2004). Also, xanthones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to the inactivation of proteins and loss of function (Stern et al., 1996). This could also explain the antimicrobial activity of compounds **1** to **6**, the antimicrobial xanthones isolated from this plant.

The results of the present study provide an important basis for the use of methanolic extract from *G. smeathmannii* for the treatment of infections associated to the microorganisms used in this study. The crude extract as well as the isolated compounds found active in this study could be useful for the development of new antimicrobial drugs. However, further pharmacological and toxicity studies currently going on in our laboratory will be necessary to confirm these hypotheses.

Acknowledgement

Author's acknowledge the technical support of the *Institut Appert* (*Paris*, France), the *A.F.R.C Reading Laboratory* (Great Britain), the *Centre Pasteur du Cameroun,* the Chinese Cooperation, the National Herbarium of Cameroon, that of Mr. Nchang Innocent of the Higher Teacher's Training College, Yaoundé, and that of Mr. Boda Maurice of the Department of Biochemistry, University of Yaoundé I.

References

Arvind, S., Reg, F.C., Enzo, A.P., 2004. Identification of antimicrobial component of an ethanolic extract of the Australian medicinal plant, *Eremophila duttonii*. Phytotherapy Research 18, 615–618.

Avril, J.L., 1997. Nouveau Dictionnaire de Bactériologie Clinique. Ellipse, Paris.

Avril, J.L., Dabernat, H., Denis, F., Monteil, H., 2000. Bactérilogie Clinique 3 ed. Ellipse, Paris.

Bacq-Calberg, C.M., Coyotte, J., Hoet, P., Nguyem-Disteche, M., 1999. Microbiologie. De Boeck & Larcier, Bruxelle, p. 338.

Carbonnelle, B., Denis, F., Marmonier, A., Pinon, G., Vague, R., 1987. Bactériologie Médicale: Techniques Usuelles. Ed SIMEP, Paris, pp. 228–282.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Review 12, 564–582.

Deachathai, S., Mahabusarakam, W., Phongpaichit, S., Taylor, W.C., Zhang, Y.J., Yang, C.R., 2006. Phenolic compounds from the flowers of *Garcinia dulcis*. Phytochemistry, 67, 464–469.

Gangoué, P.J., 2000. Résistance des bacilles Gram negatif aux antibiotiques: prévalence et caractérisation des beta-lactamases à spectres élargi à l'hôpital central de Yaoundé. Thesis of "Doctorat 3ème cycle". University of Yaounde I, Cameroon, 150 pp.

Gunasekera. 1975. Chemical Investigation of Ceylonese plants. Part 27. Extractives of *Calophyllum cuneifolium* and *Calophyllum soulattri* (Guttiferae). Journal of Chemical Society Perkin, vol. 1, p. 1505.

Gunatilaka, A.A.L., Nanayakkara, N.P.D., Sultanbawa, M.U.S., Balasubramaniam, S., 1982. Friedelin, D: A-friedo-olean-3, 21-dione and 21α-hydroxy-D: A-friedo-olean-3-one from *Kokoona zeylanica*. Phytochemistry 21, 2061–2063.

Harbone, B., 1973. Phytochemical Methods. Chapman et Hall, New York, pp. 1–150.

Herath, K., Jayasuriya, H., Ondeyka, J.G., Guan, Z., Borris, R.P., Stijfhoorn, E., Stevenson, D., Wang, J., Sharma, N., MacNaul, K., Menke, J.G., Ali, A., Schulman, M.J., Singh, S.B., 2005. Guttifaerone I, a new prenylated benzophenone from *Garcinia humulis* as liver X receptor ligand. Journal of Natural Products 68, 617–619.

Hesham, R.E.S., Ringbom, T., Torssell, K., Bohlin, L., 2003. Constituents of *Hypericum laricifolia* and their Cyclooxygenase (COX) enzyme activities. Chemistry and Pharmacology Bulletin 51, 1439–1440.

Hiroyuki, M., Emi, T., Mitsuaka, K., Yoshiyasu, F., 1996. Three xanthones from *Garcinia subellitica*. Phytochemistry 41, 629–633.

Iinuma, M., Tosa, H., Tanaka, T., Kanamaru, S., Asai, F., Kobayashi, Y., Miyauchi, K., Shimano, R., 1996. Antibacterial activity of some Garcinia benzophenone derivatives against methicillin-resistant *Staphylococcus aureus*. Biology and Pharmacology Bulletin 19, 311–314.

Iwu, M.M., Igboko, A.O., Okunji, C.O., Tempesta, S.M., 1990. Antidiabetic and aldose reductase activities of biflavanones of *Garcinia kola*. Journal of Pharmacy and Pharmacology 42, 290–292.

Kilham, C., 2004. Tamanu oil: a tropical topical remedy. J. Am. Bot. Coun., 63, 26–31.

Komguem, J., Meli, A.L., Manfouo, R.N., Lontsi, D., Ngounou, F.N., Kuete, V., Kamdem, H.W., Tane, P., Ngadjui, B.T., Sondengam, B.L., Connolly, J.D., 2005. Xanthones from *Garcinia smeathmannii* (Oliver). Phytochemistry 66, 1713–1717.

Kuete, V., Nguemeving, J.R., Penlap Beng, V., Azebaze, A.G.B., Etoa, F.-X., Meyer, M., Bodo, B., Nkengfack, A.E., 2007. Antimicrobial activity of the methanolic extracts and compounds from *Vismia laurentii* De Wild (Guttiferae). Journal of Ethnopharmacology 109, 372–379.

Locksley, H.D., Murray, I.G., 1971. Extractives from Guttiferae. Part XIX. The isolation of two benzophenones, six xanthones and two biflavonoids from the heartwood of *Allanblackia floribunda* Oliver. Journal of Chemical Society 1332–1338.

Mackeen, M.M., Ali, A.M., Lajis, N.H., Kawazu, K., Hassan, Z., Amran, M., Habsah, M., Mooi, L.Y., Mohamed, S.M., 2000. Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff. ex T. Anders. Journal of Ethnopharmacology 72, 395–402.

Meli, A.L., Komguem, J., Ngounou, N.F., Tangmouo, J.G., Lontsi, D., Ajaz, A., Iqbal, M.C., Sondengam, B.L., 2006. Antioxydant benzophenones and xanthones from the roots of *Garcinia smeathmannii*. Bulletin of Chemistry Society of Ethiopia 20, 1–6.

Meli, A.L., Komguem, J., Ngounou, N.F., Tangmouo, J.G., Lontsi, D., Ajaz, A., Iqbal, M.C., Ranjit, R., Devkota, K.P., Sondengam, B.L., 2005. Bagangxanthone A and B, two xanthones from the stem bark of *Garcinia polyantha* Oliv. Phytochemistry 66, 2351–2355.

Panthong, K., Pongcharoen, W., Phongpaichit, S., Taylor, W.C., 2006. Tetraoxygenated xanthones from the fruits of *Garcinia cowa*. Phytochemistry 67, 999–1004.

Rukachaisirikul, V., Kaewnok, W., Koysomboon, S., Phongpaichit, S., Taylor, W.C., 2000. Caged-tetraprenylated xanthones from *Garcinia scortechinii*. Tetrahedron 56, 8539–8543.

Rukachaisirikul, V., Naklue, W., Phongpaichit, S., Towatana, H.N., Maneenoon, K., 2006. Phloroglucinols, depsidones and xanthones from the twigs of *Garcinia parvifolia*. Tetrahedron 62, 8578–8585.

Sakagami, Y., Iinuma, M., Piyasena, K.G.N.P., Dharmaratne, H.R.W., 2005. Antibacterial activity of α-mangostin against vancomycin resistant Enterococci (VRE) and synergism with antibiotics. Phytomedicine 12, 203–208.

Savafi, L., Duran, N., Savafi, N., Önlen, Y., Ocak, S., 2005. The prevalence and resistance patterns of *Pseudomonas aeruginosa* in intensive care units in a university hospital. Turk. Turkey Journal of Medical Science 35, 317–322.

Sleigh, D.J., Timbury, M.C., 1998. Note on Medical Bacteriology. Churchill Livingstone, New York. 428 pp.

Stern, J.L., Hagerman, A.E., Steinberg, P.D., Mason, P.K., 1996. Phorotannin–protein interactions. Journal of Chemistry and Ecology 22, 1887–1899.

Tereschuk, M.L., Riera, M.V.Q., Castro, G.R., Abdala, L.R., 1997. Antimicrobial activity of flavonoid from leaves of *Tagetes minuta*. Journal of Ethnopharmacology 56, 227–232.

Waterman, P.G., Hussain, A.R., 1982. Major xanthones from *Garcinia quadrifaria* and *Garcinia staudtii* stem bark. Phytochemistry 21, 2099–2101.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. E & S, Livingstone, pp. 989–1008.

Zgoda, J.R., Porter, J.R., 2001. A convenient microdilution method screening natural products against bacteria and fungi. Pharmacology and Biology 39, 221–225.

Edited by JJM Meyer