SHORT REPORT

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Chemical Constituents, Antimicrobial and Cytotoxic Activities of *Hypericum riparium* (Guttiferae)

Michel F. Tala[1,2], Patricia D. Tchakam[3], Hippolyte K. Wabo[1*], Ferdinand M. Talontsi[2], Pierre Tane[1], Jules R. Kuiate[3], Léon A. Tapondjou[1] and Hartmut Laatsch[2]

[1] *Department of Chemistry, University of Dschang, P.O. Box 67, Dschang, Cameroon*

[2] *Institute of Organic and Biomolecular Chemistry, University of Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany*

[3] *Department of Biochemistry, University of Dschang, P.O. Box 67, Dschang, Cameroon*

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Abstract: Betulinic acid (**1**), 5-hydroxy-3-methoxyxanthone (**2**), 1,6-dihydroxy-7-methoxyxanthone (**3**), daucosterol (**4**), bijaponicaxanthone C (**5**), hypercalin C (**6**), 1-hydroxy-6,7-dimethoxyxanthone (**7**), cadensin D (**8**) and 5-hydroxy-1,3-dimethoxyxanthone (**9**) were isolated from the roots of *Hypericum riparium*. These compounds are reported for the first time from this plant. The extracts and two of the isolated compounds (**2** and **8)** exhibited both antibacterial and antifungal activities that varied between the microbial species (MIC = 0.97-250 µg/mL). In addition, the brine-shrimp (*Artemia salina*) lethality bioassay of compound **6** showed potent cytotoxicity with LD_{50} of 3.23 µg/mL.

Keywords: *Hypericum riparium*; antibacterial; antifungal; cytotoxic.

1. Plant source

The genus *Hypericum* occurs widely in temperate regions and have been used since ancient times as folk remedies and credited with a long list of medicinal uses, including antiviral, antimicrobial, antifungal, antitumor, analgesic, anxiolytic, sedative and for the treatment of neurological disorders and depression [1]. These effects have inspired investigations of secondary metabolites from *Hypericum* species. As results, various classes of bioactive components mainly xanthones, phloroglucinols, flavonoids, naphtodianthrones and essential oils have been isolated and identified [1-5]. In continuation of our investigations on plants of *Hypericum* genus [6,7] we herein report the isolation of nine compounds from the roots of *Hypericum riparium*, together with the antimicrobial and cytotoxic activities of extracts and some isolated compounds. The roots of *H. riparium* A. Chev. were collected in June 2010 at Mount Bamboutos, West Region of Cameroon. Identification was done at the Cameroon National Herbarium, Yaounde, where a voucher specimen (No 33796 HNC) has been deposited.

* Corresponding author : E-Mail : hkamdemw@yahoo.ca; Phone: +237 77 74 07 53; Fax: +237 33 45 17 35

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2. Previous studies

To the best of our knowledge, there are no phytochemical and biological reports on *H. riparium.*

3. Present study

Chromatography of the EtOAc-soluble portion of the CH_2Cl_2-MeOH (1:1) extract of the roots of *H. riparium* afforded betulinic acid (**1**), 5-hydroxy-3-methoxyxanthone (**2**), 1,6-dihydroxy-7-methoxyxanthone (**3**), hypercalin C (**6**) and cadensin D (**8**). EtOAc-insoluble residue was subjected to repeated column chromatography to yield daucosterol (**4**), bijaponicaxanthone C (**5**), 1-hydroxy-6,7-dimethoxyxanthone (**7**) and 5-hydroxy-1,3-dimethoxyxanthone (**9**).

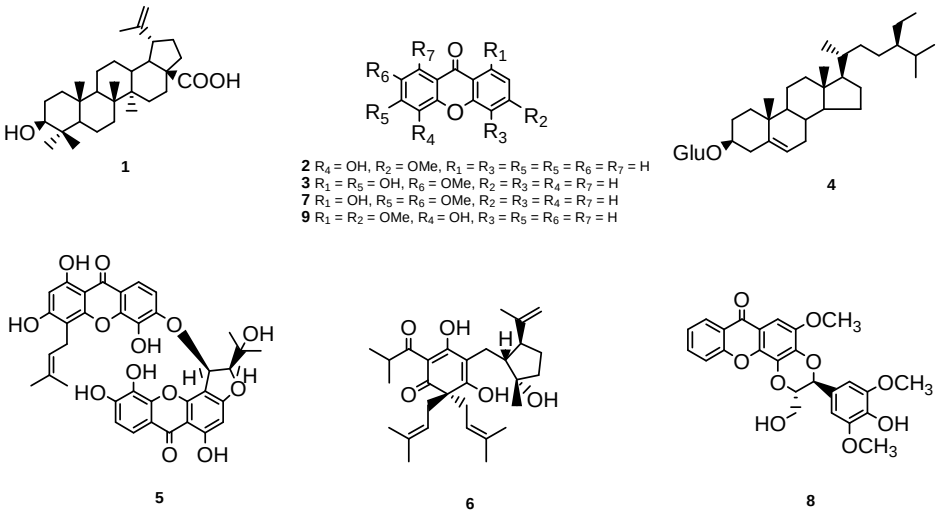

Figure 1. Compounds **1-9** isolated from *H. riparium*

Cytotoxicity assay [8]: The EtOAc extract and compound **6** showed potent cytotoxic activity in the brine-shrimp (*Artemia salina*) lethality bioassay. It was evident from the results that compound **6** was significantly lethal with LD_{50} value of 3.23 µg/mL (Table 1). The other isolated compounds did not show any activity in this test.

Table 1. Cytotoxic activity of the EtOAc extract and compound **6** against *Artemia salina*[a]

	Doses (µg/mL)	3.12	6.25	10.0	12.5	25	50	100	LD_{50} (µg/mL)
	EtOAc extract	0	0	100	100	100	100	100	<10
% Deaths	**6**	0	47	100	100	100	100	100	3.23
	Actinomycin D[b]	100	100	100	100	100	100	100	0.02

[a] 10 µL of 10 µg/mL for each compound was tested. Mortality in % is determined after 24 h. 100% mortality: high active sample; [b] Reference drug

Antimicrobial activities [9]: The crude CH_2Cl_2-MeOH (1:1) extract, EtOAc-soluble fraction, EtOAc-insoluble fraction as well as compounds **2** and **8** exhibited both antibacterial and antifungal activities that varied between the microbial species (MIC = 0.97-250 µg/mL) (Table 2). The EtOAc extract was more active than the crude extract and the EtOAc-insoluble portion. Compound **2** has a large spectrum of activity whereas compound **8** showed a strong activity against *Candida lusitaniae* (MIC = 3.90 µg/mL), compared to that of the reference drug (MIC = 7.81 µg/mL). Compound **6** was inactive against all the tested microorganisms. This is the first report on the antibacterial and antifungal activities of compound **2** and the antifungal activity of compound **8** against *Candida lusitaniae*. Compound **2** was recently found to show a weak activity on the multidrug-resistant W2mef laboratory strain, and a field isolate (SHF4) of *Plasmodium falciparum* [7].

Table 2. Inhibition parameters (MIC, MBC and MFC) of the extracts and compounds **2**, **6** and **8** (µg/mL)

Microorganism	Parameters	Tests substances						
		CE	EASF	EAIF	**2**	**6**	**8**	Reference antibiotics[c]
Bacteria								
Staphylococcus aureus	MIC	1000	31.5	500	-	-	-	15.32
	MBC	-	250	-	-	-	-	15.32
Escherichia coli	MIC	500	125	125	-	-	125	1.95
	MBC	-	1000	500	-	-	-	3.9
Shigella flexneri	MIC	1000	1000	-	0.97	-	-	1.95
	MBC	-	-	-	1.95	-	-	7.81
Salmonella typhi	MIC	1000	15.62	62.5	1.95	-	-	1.95
	MBC	-	250	250	1.95	-	-	1.95
Proteus mirabilis	MIC	250	62.5	62.5	62.5	-	-	3.90
	MBC	-	250	250	62.5	-	-	7.81
Enteroccocus faecalis	MIC	-	1000	-	0.97	-	125	1.95
	MBC	-	-	-	1.95	-	-	1.95
Yeast								
Candida lusitaniae	MIC	250	500	1000	-	-	3.90	7.81
	MFC	250	1000	-	-	-	3.90	15.32
Cryptococcus neoformans	MIC	250	-	250	250	-	-	1.95
	MFC	-	-	-	250	-	-	3.90
Candida glabbrata	MIC	250	-	-	-	-	-	1.95
	MFC	1000	-	-	-	-	-	3.90
Candida parapsilosis	MIC	125	1000	250	-	-	-	31.25
	MFC	1000	-	-	-	-	-	-
Candida krusei	MIC	-	-	-	-	-	-	31.25
	MFC	-	-	-	-	-	-	-

-: Not active (>1000 µg/mL for crude extracts, >250 µg/mL for compounds tested on yeasts and 125 µg/mL for compounds tested on bacteria); c: Ciprofloxaxin for bacteria and nystatin for yeasts; CE: crude CH_2Cl_2-MeOH (1:1) extract; EASF: EtOAc-soluble fraction; EAIF: EtOAc-insoluble fraction

Chemotaxonomic significance: The present study reports the isolation of four xanthones (**2**, **3**, **7** and **9**), one prenylated bisxanthone (**5**), and one xanthonolignoid (**8**) together with one phloroglucinol (**6**), one triterpene (**1**) and one steroid (**4**) for the first time from the roots of *H. riparium*. Oxygenated xanthones, xanthonolignoids and phloroglucinol are common to the Guttiferae family and particularly in the genus *Hypericum* [3-6]. 1-Hydroxy-6,7-dimethoxyxanthone (**7**) and cadensin D (**8**) have been reported from *H. perforatum* and *H. subalatum* respectively [4,5]. This is the second report of compounds **5** and **6** in the genus. Bijaponicaxanthone C (**5**) has been previously isolated from *H. japonicum* [10], while hypercalin C (**6**) was obtained from *H. calycinum* [11]. Interestingly, 1,6-dihydroxy-7-methoxyxanthone (**3**) and 5-hydroxy-1,3-dimethoxyxanthone (**9**) are reported here for the first time from the genus *Hypericum.* Coumpound **9** has been isolated from the genera *Kielmeyera* and *Garcinia* of the same family [12,13], while compound **3** was isolated only once from *Poeciloneuron pauciflorum* [14]. We have recently reported the isolation and characterization of several xanthones including 5-hydroxy-3-methoxyxanthone (**2**) from the stem bark of *H. lanceolatum* [6]. *H. riparium* is one of the six *Hypericum* species found in Cameroon [6]. Thus, isolation of compounds **2**, **3** and **9** in the present investigation might be a useful contribution to the chemotaxonomic studies of the Cameroonian *Hypericum* species as well as of the Guttiferae family.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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