Antibacterial activity of chalcone and dihydrochalcone compounds from Uvaria chamae roots against multidrug resistant bacteria

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Dougnon TV.1, Koudokpon H.1-2 #, Armstrong N.2 #,, Fah L.1, Hounsa E. 1, Bankole HS.1, Loko F.1, Chabriere E.2, Rolain JM2.

1Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 PO Box 2009 Cotonou, Benin; 2Aix Marseille Université, IRD, APHM, MEPHI, IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France

INTODUCTION

Uvaria chamae is originated from tropical forests in the west and centre of Africa. Its fruit is edible and its roots show great interest throughout the world. Many activities have been assigned to this plant so far: antiparasitic (Adelodun *et al.*, 2013; Fall *et al.*, 2003), antiplasmodial (Okokon *et al.*, 2006), antidiabetic (Olorunnisola *et al.*, 2016), anti-diarrheal (Ambe *et al.*, 2015), antifungal (Kongstad *et al.*, 2015;), anti-inflammatory (Omajali *et al.*, 2011) or antimalarial (Oke *et al.*, 2013). In Benin, *Uvaria chamae* roots are sold as antidiabetic (Fah *et al.*, 2013) and for the treatment of infections (Koudokpon *et al.*, 2017). Yet, no study has identified the compounds responsible for this antimicrobial activity. This present study now addresses the *in vitro* antimicrobial activity of *Uvaria chamae* root extracts and reveals the related compounds.

Material and methods

Root collection and extraction were done in the district of Cocotomey (Abomey calavi, Bénin). Samples were stored at room temperature before further extraction.

Antimicrobial activity of root extract was evaluated using Mueller Hinton agar culture plates and the agar well diffusion method (Balouiri et al., 2016). Each activity test was performed three times. (Tsirinirindravo & Andrianarisoa, 2009).

Root extracts were heated at 90 °C for 30 minutes. Concentrated extracts were diluted in water pH 2 and 10 adjusted with NaOH or HCl.

100 µl of concentrated ethanol extract (100 mg/mL) was first evaporated under a stream of nitrogen at 40 °C. The chemicals were then dissolved in 100 µl of water at pH 10 and the solution centrifuged at 16000 g. 10 µl of supernatant was injected on a UHPLC-UV chromatography system to separate the compounds.

The fractions found to be active against selected bacteria were then pooled for subsequent LC/MS analysis using an Acquity I-Class chromatography system connected to a Vion IMS QTOF ion-mobility high resolution time-of-flight mass spectrometer (Waters).

Results

Antibacterial activity

First of all, culture inhibition from *Uvaria chamae* root extracts was only observed for Gram-positive cocci including the following tested strains *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium* (Table 1).

Table 1. Inhibition diameters measured for root extracts and antibiotic discs

Strain name	Reference	Uvaria chamae roots			Antibiotic discs (controls)			
		Water Extract	Ethanol-Water Extract	Ethanol Extract	colistin	imipenem	vancomycin	mupirocine
S. aureus	ATCC 259223	0	9.0±0.6 a,b	27.7±0.9 a,b	-	-	20.3±0.9	30.7±0.7
S. aureus Méti R	Clinical isolate	0	9.0±0.6 a,b	25.0±0.6 a,b	-	-	20.67±0.3	31.3±1.3
S. aureus Mupi R	Clinical isolate	0	9.0±0.6 a	28.3±0.3 a	-	-	19.3±0.3	0
E. faecalis Van A	Clinical isolate	0	15.7±0.3	30.7±0.7	-	-	0	-
E. faecium Van A	Clinical isolate	0	15.7±0.3	30.3 ±0.3	-	-	0	-
E. faecium Van B	Clinical isolate	0	15.7±0.3	28.3±0.3	-	-	0	-
E. coli	ATCC 25922	0	0	0	20	28	-	-
E. coli BLSE	Clinical isolate	0	0	0	20	28	-	-
K. pneumoniae BLSE	Clinical isolate	0	0	0	20	28	-	-
E. cloacae BLSE	Clinical isolate	0	0	0	20	28	-	-
P. aeruginosa Vim-2	Clinical isolate	0	0	0	20	0	-	-

Chemical properties

First tests indicated that the activity of extracts was maintained after heating, protein digestion and adjustment towards alkaline pH values. Consequently, active compounds were not proteins. Furthermore, high chromatographic retentions using a phenyl-hexyl column suggested structures with aromatic rings.

Chromatograms showed several intense peaks at the end of the elution gradient (Figure 1), corresponding to antimicrobial active retention times between 5 and 7 minutes.

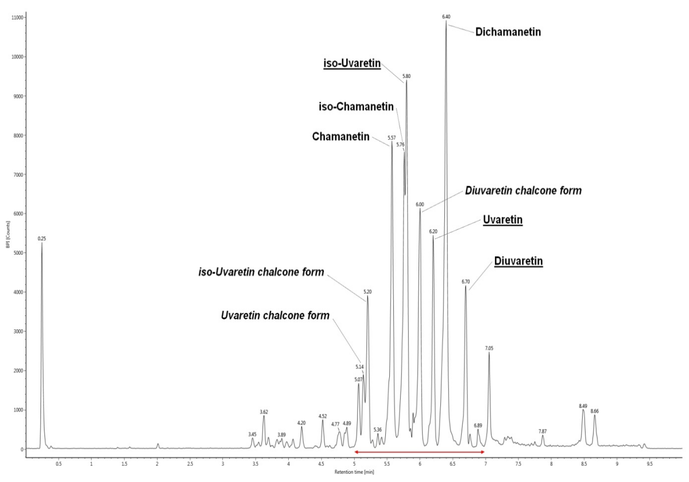

Figure 1: Base Peak Intensity (BPI) extracted LC/MS chromatogram of the pooled active fractions. Identified peaks are labelled and the red line indicates the antimicrobial activity.

LC/MS identifications

High definition LC-MS analysis of the pooled active collected fractions revealed the presence of flavonoid compounds that correspond to the supposed chemical functions (Table 2).

Table 2. MIC, MBC and Antibacterial Power (AP) for selected ethanol plants extracts

	Uvaria chamae (ethanolic extract)		
	MIC	MBC	AP
Staphylococcus aureus ATCC 25923	0,0046	0,0093	1*
Staphylococcus aureus Méti R	0,0046	0,018	2*
Staphylococcus aureus Mupi R	0,0046	0,037	2*
Enterococcus faecalis Van A	0,0023	0,037	4
Enterococcus faecium Van A	0,0023	0,075	4
Enterococcus faecium Van B	0,0023	0,075	4

Compounds identifications were confirmed with parent mass errors below 1 ppm (error according to chemical formula) and at least 3 predicted fragments below 10 mDa error. The corresponding dihydrochalcone structures are detailed in Figure 2.

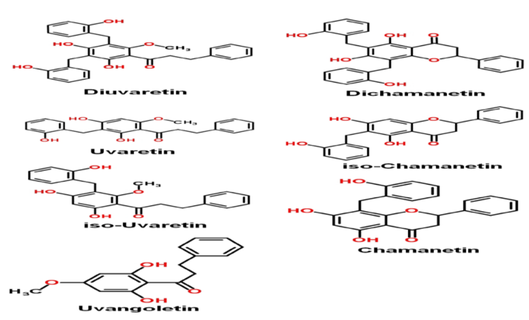

Figure 2: dihydrochalcone structures identified from the active fractions

Latest chalcone structures are detailed in Figure 3. They present same skeleton structures as Uvaretin family compounds containing one unsaturation (minus two hydrogenes), probably a double bond on the chalcone chain.

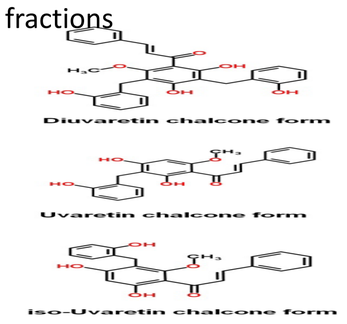

Figure 3: new chalcone structures identified from the active fractions

Discussion

First, the evaluation of the antimicrobial activity of Uvaria chamae root extracts indicated high activity for ethanol extracts according to previously described standard diffusion methods (Bauer et al., 1966; Tsirinirindravo & Andrianarisoa, 2009). This activity was only measured against Gran-positive cocci and none was visualized against any of the tested Gram-negative strains. A previous study presented similar results (Oluremi et al., 2010),

Nowadays, vancomycin or mupirocine antibiotics are often the last treatment solution against multi-drug resistant bacteria. In comparison with these latest antibiotics, Uvaria chamae ethanol root extracts showed here a more extensive inhibition activity, therefore indicating a good potential as an efficient antibiotic.

The identification of several similar chemical structures consolidates here the identification of some suspected structures wich are all part of the flavonoids class within the polyketide lipid category. All of them have a common carbon backbone, with varying numbers of hydrobenzyl functional groups, that could explain the similar activity in multiple tested fractions.

Dihydrochalcone structures were well described previously (Parmar et al., 1993) and are all associated to Uvaria chamae according to the literature.

Several studies already spotted the implication of chalcones (Oldoni et al., 2011; patel et al., 2015; Shah & Goswami, 2013) and dihydrochalcones (Lavoie et al., 2013; Simard et al., 2015) as antimicrobial chemicals.

As presented here, Khan & Asiri (Khan & Asiri, 2017) also found a strong activity of chalcones against Gram-positive cocci (*S aureus* and *S pyogenes*) compared to Gram-negative bacteria (*S typhimurium* and *E coli*). Antimicrobial activity of dihydrochalcones form *Populus balsamifera* tree buds was also reported before (Chassion, 2012)

Conclusion

Taken together, these new findings affirm that traditional medicine using the root of *Uvaria chamae* is of great value for the treatment of microbial infections. This knowledge about flavonoid active ingredients will be helpful for future medication and research in this area. Furthermore, such chalcone and dihydrochalcone compounds show a great potential in the challenging treatment of multidrug resistant bacteria. Finally, further chemical description and antimicrobial activity measurements of each individual detected compounds are important perspectives that should be addressed in the future.