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Preliminary evidences of the direct and indirect antimicrobial activity of 12 plants used in traditional medicine in Africa

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Abstract In a world of increasing resistance to current antibiotics, search of novel therapeutic options is urgently needed. The aim of this work was to screen plant crude extracts for direct or indirect (inhibition of resistance) antimicrobial activity. Four crude extracts from 12 plants traditionally used in Africa for the treatment of infections were obtained by successive extraction with hexane, dichloromethane, ethyl acetate, and methanol. All extracts were tested against *Staphylococcus aureus* MRSA ATCC33591 [resistant to β-lactams by production of β-lactamases and of a modified PBP target (PBP2a)]. Direct antimicrobial activity was tested by determination of Minimal Inhibitory Concentrations (MIC), and indirect activity, by determining interactions between antibiotics and extracts using checkerboard titration and calculation of Fractional Inhibitory Concentration Index (FICI; synergy: FICI $\leq$ 0.5; additivity: FICI $\leq$ 1). Combined antibiotics were ampicillin (sensitive to resistance mediated by β-lactamases and PBP2a) and oxacillin (sensitive to resistance mediated by PBP2a only). The dichloromethane extract of *Vitellaria paradoxa* leaves, the methanol extracts of *Vitellaria paradoxa*, *Cola gigantea* leaves and twigs, and of *Tapinanthus bangwensis* aerial parts showed direct antimicrobial activity (MIC 250–500 mg/L). The methanol extracts of *Vitellaria paradoxa* and *Cola gigantea* leaves and twigs showed additive or synergistic effects with oxacillin and ampicillin on MRSA ATCC33591 (FICI 0.28–1), suggesting a possible inhibition of PBP2a. The methanol extract of *Tapinanthus bangwensis* aerial parts and *Anchomanes difformis* roots improved the activity of ampicillin only (FICI 0.38–1), suggesting β-lactamase inhibition. Polyphenols and particularly tannins were shown to be responsible for these last effects, at least partially for *Vitellaria paradoxa*. These data need further research aiming at identifying the active compounds in these extracts.

Keywords Plant extracts · Resistance modifying agent · MRSA · β-Lactams · Tannins

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Introduction

Infections caused by multi-drug resistant bacteria are an increasing problem due to the emergence and propagation of microbial drug resistance and the lack of development of new antimicrobials.

Staphylococcus aureus is a major cause of hospitally-acquired infections, with β-Lactam antibiotics often considered as a first line of treatment. However, widespread use of this class of antibiotics has led to an

increasing prevalence of bacterial resistance. The main mechanisms of β-lactams resistance consist in (a) the production of β-lactamases inactivating the antibiotics and (b) the production of PBP2a, an additional penicillin-binding protein with decreased affinity for methicillin and most other β-lactam drugs, the production of which is associated to the so-call MRSA (Methicillin-resistant *Staphylococcus aureus)* phenotype (Tenover 2006). MRSA is one of the most prominent pathogens known to induce a severe threat to human health (Lowy 1998). Recent epidemiological studies indicate that the prevalence of MRSA is high over the world posing a significant public health challenge (Hadler et al. 2012; Johnson 2011).

Therapeutic options available for treating MRSA infections have become seriously limited. Indeed, MRSA have also acquired resistance mechanisms to most of the other available antibiotic classes (Miller et al. 2014; Sandora and Goldmann 2012). Moreover, the rapid widespread and emergence of resistance to newly introduced antimicrobial agents indicates that new families of antimicrobial agents will have a short life expectancy (Coates et al. 2002). Although ceftaroline, telavancin, tedizolid, dalbavancin, and oritavancin have been recently marketed as drugs to treat MRSA infections, the development of new and original strategies to fight them is still needed (Rodvold and McConeghy 2014).

A useful alternative approach consists in inhibiting resistance mechanisms, which has already been widely exploited with β-lactamase inhibitors (Drawz and Bonomo 2010). During the last decade, a few studies reported that plant extracts can contain antimicrobial compounds and/or substances enhancing the in vitro activity of certain antibiotics against multi-drug resistant strains of *Staphylococcus aureus* (Abreu et al. 2012; Chanda and Rakholiya 2011; Gibbons 2004, 2005; Hemaiswarya et al. 2008; Sibanda and Okoh 2007; Taylor 2013).

In this study, we screened 12 plants traditionally used in Africa to treat diverse infections for their direct antimicrobial activity against a MRSA reference strain. We also determined their indirect antimicrobial activity in combination with two β-lactams (ampicillin and oxacillin). For each plant species, twigs, leaves or roots were extracted with hexane, dichloromethane, ethyl acetate and methanol. Crude extracts were also evaluated for their cytotoxicity on two mammalian cell lines.

Materials and methods

Plant material

Most of the plant materials (leaves and twigs (=aerial parts), and roots) were collected from the South of Benin, especially from Abomey–Calavi (South–West) to the border area with Nigeria (South-East) between September 2006 and August 2012. *Anthocleista schweinfurthii* was collected in Kinshasa (Democratic Republic of the Congo) in August 2007 and *Spirospermum penduliflorum,* in December 2009 in the Eastern Region of Madagascar. Voucher specimens were identified and deposited at the Herbier National of Abomey-Calavi University in Benin, at the herbarium of the "*Institut Malgache de Recherches Appliquées*" (IMRA) or at the Herbarium of the National Botanic Garden of Belgium, at Meise (see Table 1).

Preparation of crude plant extracts and tannins free fractions

Dried leaves, twigs, or roots (50–100 g) were extracted using a soxhlet apparatus with four solvents of increasing polarity (hexane, dichloromethane, ethyl acetate and methanol) for 8 h each. The quantity of solvent used for each extraction was 250 mL. The extracts obtained were then evaporated to dryness under reduced pressure with a rotary evaporator at a temperature of 30 °C. Yields for each extraction are indicated in Table 2.

Active methanolic extracts were cleared of tannins with a polyamid column (Polyamid CC 6, Macherey—Nagel) according to the method described by Houghton and Raman (1998). 20 g of polyamid were swollen in 200 mL of water during one night. A column was filled with the gel. After one day, the column was conditioned with two column volumes of each solvent used in the elution. 500 mg of extract solubilized in a minimum of solvent (mixture MeOH/H_2O 50:50) were deposed on the column. For the elution, we used MeOH, a mixture of MeOH/H_2O 50:50, ethyl acetate and finally dichloromethane.

Table 1 Plant species under study

Botanical name	Family	Voucher specimen number	Collecting place	Traditional uses
Allophylus africanus P. Beauv	Sapindaceae	AP2131	Benin	To treat headaches, migraines, colic, fever, conjunctivitis and other eye troubles, wounds, infections, diarrhea and malaria[a,b,c,d]
Anchomanes difformis (Blume) Engl.	Araceae	AP2132	Benin	As diuretic, to treat diabetes, oral and anal lesions, tuberculosis and malaria[a]
Anthocleista schweinfurthii (Gilg.)	Gentianaceae	BR0000009752597	Kinshasa	To treat diabetes, malaria, stomach diseases, ovarian problems, veneral disease, bronchitis, fever, abscesses, malaria and to facilitate wound healing[e,f]
Cassia sieberiana DC	Caesalpiniaceae	BR S.P. 848100	Benin	To treat fever, jaundice, stomach ache, gonorrhea, piles, ulcers, abscesses and malaria[g,h]
Cissampelos mucronata A Rich.	Menispermaceae	AA 6347/HNB	Benin	To treat wounds, indigestion, schistosomiasis, infections, malaria[i]
Cola gigantea A. Chev. Var. gigantea	Sterculiaceae	AP2133	Benin	To treat sores, skin infections and other inflammatory conditions including pains[j]
Holarrhena floribunda (G. Don) Durand & Schinz	Apocynaceae	AP2128	Benin	As febrifuge and tonic, to treat dysentery, malaria, female sterility, tripanosomiasis, abdominal pains, nausea, indigestion, diarrhea[k,l,]
Protea elliotii C. H. Wright	Proteaceae	AA 6354/HNB	Benin	To treat carious teeth, hemorrhoids, sores, skin diseases[m,n]
Schrankia leptocarpa DC.	Mimosaceae	Houngnon 954b	Benin	To treat eruptive fever, hypertension, jaundice, abdominal pains, hiccup and malaria[a]
Spirospermum penduliflorum Thouars	Menispermaceae	AML13	Madagascar	To treat malaria, pulmonary tuberculosis, vomiting, colic, liver complains and as cardiac tonic[p]
Tapinanthus bangwensis (Engl. & Krause) Danser	Loranthaceae	AP2242	Benin	To treat snake bites, disorders in female reproductive system, cancer, arthritis, rheumatism, hypertension, asthma, diabetes, wounds, skin infections[n]
Vitellaria paradoxa C.F. Gaertn. ssp. *paradoxa*	Sapotaceae	AP2130	Benin	To treat hypertension, wound infections, skin infections, dysentery, diarrhea, gastrointestinal tract infections, jaundice, hemorrhoids, fever[m,n,o]

[a]Adjanohoun et al. (1989), [b] Balogun et al. (2014), [c] Oladosu et al. (2013), [d] Oladosu et al. (2015), [e] Mbouangouere et al. (2007), [f] Mezui et al. (2015), [g] Ahua et al. (2007), [h] Zerbo et al. (2011), [i] Nondo et al. (2011), [j] Agyare et al. (2012), [k] Nwodo et al. (2007), [l] Bogne et al. (2007), [m] Tagne et al. (2014), [n] Jiofack et al. (2010), [o] Ayankunle et al. (2012), [p] Schmelzer and Gurib-Fakim (2008)

TLC analysis

Methanolic extracts were resolubilized in methanol at a concentration of 10 mg/mL. 5 µL were then spotted on a TLC Silica gel 60 F_{254} plate (Merck, Darmstadt, Germany). The mobile phase consisted in a mixture of butanol/acetic acid/H_2O (14:5:5). Revelation was done with chlorhydric vanillin prepared according to the method described by Wagner and Bladt (1996).

Bacteria, cells and media

Crude extracts were tested for their direct and indirect antibacterial activity against *Staphylococcus aureus* ATCC33591 (MRSA; obtained from the American Type Culture Collection). This reference strain is resistant to β-lactams by two main mechanisms, namely the production of β-lactamases and of targets PBP2a.

Two cell lines were used in parallel, and cultivated in a humidified atmosphere with 5 % CO_2 at 37 °C. The J774 macrophage-like cell line, derived from BALB/c murine reticulum cell sarcoma, was cultivated in RPMI 1640 medium (Life Technologies, Paisley, UK) containing 2 mM L-glutamine supplemented with 10 % heat-inactivated fetal bovine serum (Life Technologies) and penicillin–streptomycin (100 UI/mL). The human normal fibroblast cell line, WI38, was cultivated in DMEM medium (Life Technologies) containing 4 mM L-glutamine, 1 mM sodium pyruvate supplemented with 10 % heat inactivated fetal bovine serum (Life Technologies) and penicillin–streptomycin (100 UI/mL).

Antibiotics and antibiotic susceptibility testing

Ampicillin (potency 87.99 %) and oxacillin (potency 90 %) (Sigma-Aldrich, St Louis, MO) were used as tested antibiotics.

Stock solutions of crude extracts were prepared at 20 mg/mL in dimethylsulfoxide (DMSO) and then serially diluted in the medium to obtain a final concentration range of 500–7.81 mg/L. The highest concentration of solvent to which the bacteria were exposed was 2.5 %, which was shown in preliminary experiments to have no effect on the growth of bacteria.

Minimum Inhibitory Concentrations (MIC) and Fractional Inhibitory Concentration Indices (FICI) were determined by the checkerboard method in cation-adjusted Mueller–Hinton broth (CA-MHB) (Buyck et al. 2015; Eliopoulos and Moellering 1996). In a 96-well plate, the β-lactam antibiotic in the combination was serially diluted starting from a final concentration of 2 × MIC along the ordinate. The crude extract was serially diluted along the abscissa using 500 mg/L as the highest final concentration. After that, a bacterial suspension (final inoculum 0.5 10^6 to 1 10^6 CFU/mL) was added to all wells.

After 20 h of incubation at 37 °C, the MICs of antibiotics and crude extracts were determined as the lowest concentration that completely inhibited the growth of the organism.

Interactions between antibiotics were then evaluated using the FIC indices, calculated as the sum of the Fractional Inhibitory Concentrations (FICs) as follows: FICI = FIC A + FIC E, where FIC A is MIC of the antibiotic in the combination/MIC of the antibiotic alone and FIC E is MIC of the extract in the combination/MIC of the extract alone (Bonapace et al. 2002). The combination was considered as synergistic for FICI $\leq$ 0.5, additive for 0.5 $<$ FICI $\leq$ 1, indifferent for 1 $<$ FICI $\leq$ 4 and antagonistic for FICI $>$ 4 according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST 2000). To facilitate readings, bacterial growth was detected using resazurin, a blue phenoxazin dye that is reduced by viable bacteria in the pink fluorescent compound resorufin (Sarker et al. 2007). 30 µL of a 0.02 % resazurin (Sigma-Aldrich) solution in CA-MHB was added to each well. Plates were then incubated at 37 °C for 1 h in the dark. MIC corresponded to the lowest concentration of antibiotic or extract for which the well color did not turn to pink.

Cytotoxicity assay

The cytotoxicity of the extracts for J774 and WI38 cells was evaluated as described by Stevigny et al. (2002), using tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphényltetrazolium bromide] (Sigma-Aldrich) colorimetric method based on the cleavage of the reagent by mitochondrial dehydrogenase in viable cells (Mosmann 1983). Camptothecin (Sigma-Aldrich) was used as a positive cytotoxic reference. Stock solutions of crude extracts at 20 mg/mL in DMSO were diluted in medium with a final concentration range of 100–0.01 mg/L. The cytotoxicity of solvents was tested in parallel and found to be negligible at the highest concentration used for extracts (0.5 %). Each extract was tested in nine serial dilutions in 96-well microtiter plates.

Results and discussion

Fifty-six extracts were obtained from the 12 plants under study. In vitro direct and indirect antimicrobial and cytotoxic activities of these extracts are summarized in Table 2.

Table 2 In vitro direct and indirect antibacterial activity and cytotoxicity of the selected plant extracts (n = 3)

Plants species	Part studied[a]	Extract	Yield (%)	Antibacterial activity against MRSA ATCC33591			Cytotoxicity (IC_{50}, mg/L) average ± standard deviation	
				MIC (mg/L)	FICI AMPI	FICI OXA	WI38	J774
Allophylus africanus	AP	HEX	2.33	>500	0.63–1	1	77.99 ± 8.89	97.81 ± 3.10
		DCM	1.26	>500	1	1–2	60.69 ± 6.18	19.79 ± 2.24
		AcOEt	0.89	>500	1	1–2	>100	62.43 ± 6.27
		MeOH	5.07	>500	1	1–2	>100	>100
Anchomanes difformis	RT	HEX	0.46	>500	0.18–1	0.60–1	>100	76.4 ± 9.3
		DCM	0.56	>500	1	1	>100	>100
		AcOEt	0.71	>500	1	1–2	>100	>100
		MeOH	8.93	>500	0.38–1	1	>100	>100
Anthocleista schweinfurthii	RB	HEX	0.53	>500	0.62–1	1–2	66.06 ± 7.75	23.17 ± 3.36
		DCM	0.52	>500	0.75–1	1–2	67.26 ± 2.16	47.10 ± 2.00
		AcOEt	0.63	>500	0.75–1	1–2	>100	91.92 ± 7.57
		MeOH	6.91	>500	1	1–2	>100	>100
Cassia sieberiana	AP	HEX	1.77	>500	1	1	88.36 ± 0.89	44.54 ± 5.39
		DCM	1.35	>500	1–2	1	63.42 ± 7.29	41.31 ± 8.92
		AcOEt	1.32	>500	1–2	1	>100	>100
		MeOH	14.54	>500	1	1	>100	>100
Cissampelos mucronata	AP	HEX	1.65	>500	1	1–2	29.17 ± 1.43	31.07 ± 8.64
		DCM	0.82	>500	1	1–2	65.68 ± 6.91	18.32 ± 2.31
		AcOEt	0.78	>500	1	1–2	45.16 ± 3.78	19.24 ± 0.30
		MeOH	7.91	>500	1	1–2	99.57 ± 0.54	35.46 ± 6.15
Cola gigantea	TW	HEX	0.84	>500	1	1–2	42.69 ± 8.49	35.06 ± 0.39
		DCM	0.45	>500	1	1–2	32.85 ± 8.95	31.54 ± 3.32
		AcOEt	0.42	>500	1	1–2	>100	82.93 ± 3.48
		MeOH	12.34	500	0.5–1	0.28–1	>100	88.22 ± 6.31
	LF	HEX	3.07	>500	1	1–2	>100	56.92 ± 6.94
		DCM	1.18	>500	1	1–2	>100	57.69 ± 2.69
		AcOEt	0.59	>500	1	1–2	>100	80.48 ± 9.83
		MeOH	12.81	500	0.38–1	0.31–1	>100	>100
Holarrhena floribunda	AP	HEX	3.96	>500	1	1–2	28.24 ± 0.68	33.04 ± 1.82
		DCM	2.02	>500	1	1–2	28.26 ± 1.64	36.98 ± 5.62
		AcOEt	1.54	>500	1	1–2	25.80 ± 4.65	35.13 ± 1.23
		MeOH	16.56	>500	0.56–1	1	16.53 ± 0.43	22.81 ± 0.35
Protea elliotii	AP	HEX	1.25	>500	0.75–1	1–2	83.16 ± 6.48	27.45 ± 1.52
		DCM	0.89	>500	1	1–2	88.63 ± 3.86	50.61 ± 1.37
		AcOEt	2.7	>500	0.50–1	1	>100	>100
		MeOH	16.56	>500	0.75–1	1	>100	>100
Schrankia leptocarpa	AP	HEX	1.98	>500	0.62–1	1	>100	>100
		DCM	0.99	>500	1	1	>100	51.26 ± 6.75
		AcOEt	0.88	>500	1	1	>100	>100
		MeOH	14.51	>500	1	1	>100	>100

Table 2 continued

Plants species	Part studied[a]	Extract	Yield (%)	Antibacterial activity against MRSA ATCC33591			Cytotoxicity (IC_{50}, mg/L) average ± standard deviation	
				MIC (mg/L)	FICI AMPI	FICI OXA	WI38	J774
Spirospermum penduliflorum	AP	HEX	2.71	>500	1	1–2	61.10 ± 7.38	31.66 ± 3.11
		DCM	1.76	>500	0.56–1	1–2	10.10 ± 0.20	4.82 ± 0.67
		AcOEt	0.91	>500	1	1–2	75.30 ± 8.45	21.63 ± 4.93
		MeOH	10.18	>500	1	1–2	>100	32.03 ± 6.92
Tapinanthus bangwensis	AP	HEX	2.94	>500	1	1	>100	71.28 ± 2.93
		DCM	0.68	>500	0.75–1	1	28.89 ± 1.98	29.63 ± 8.64
		AcOEt	0.87	>500	1	1	85.1 ± 7.58	63.02 ± 2.02
		MeOH	10.24	500	0.38–1	1	>100	>100
Vitellaria paradoxa	TW	HEX	3.16	>500	1	1–2	86.83 ± 9.23	72.56 ± 1.76
		DCM	0.39	>500	1	1	25.85 ± 0.92	33.92 ± 0.88
		AcOEt	0.33	>500	1	1	>100	>100
		MeOH	7.26	500	0.38–1	0.38–1	95.90 ± 5.80	47.74 ± 1.82
	LF	HEX	4.62	>500	1	1	53.86 ± 2.89	43.55 ± 2.90
		DCM	1.83	250	0.14–1	0.51–1	7.17 ± 2.93	27.56 ± 0.13
		AcOEt	1.25	>500	0.63–1	1–2	>100	72.31 ± 0.42
		MeOH	16.06	500	0.50–1	0.50–1	>100	47.74 ± 11.18
Camptothecin				nd	nd	nd	0.03 ± 0.003	0.4 ± 0.2

MRSA33491 methicillin resistant *Staphylococcus aureus*, *J774* macrophage-like murine cells, *WI38* human normal fibroblast cell, *nd* not determined

[a] Plant part used: *LF* leaves, *TW* twigs, *RT* roots, *AP* aerial parts (leaves + twigs), *RB* root bark

Concerning the direct antimicrobial activity on MRSA ATCC33591, 6 extracts had an MIC lower or equal to 500 mg/L, namely the methanol extracts of *Vitellaria paradoxa* (leaves and twigs), *Cola gigantea* (leaves and twigs), and *Tapinanthus bangwensis* aerial parts and the dichloromethane extract from the leaves of *Vitellaria paradoxa*. According to Kuete (2010), those crude extracts may be considered as moderately active ($100 < MIC \leq 625$ mg/L) and they thus may contain very interesting pure compounds.

To determine the indirect antimicrobial activity, we tested the combinations of the crude extracts with two β-lactams selected to allow us to distinguish between a reversion of two mechanisms of resistance. Thus, while both ampicillin and oxacillin are sensitive to PBP2a-mediated resistance, oxacillin resists to the action of β-lactamases, thanks to a steric hindrance on its lateral chain (Barber and Waterworth 1964; Kalant 1965). A decrease in the MIC of both antibiotics can thus be interpreted as denoting an influence of the extract on PBP2a-mediated resistance while a decrease in the MIC of ampicillin only would be indicative of an inhibition of the β-lactamases activity.

Globally, 14 extracts, namely the hexane extracts from the aerial parts of *Allophyllus africanus, A. schweinfurthii, Protea elliotii* and *Shrankia leptocarpa,* the dichloromethane extracts from *A. schweinfurthii, S. penduliflorum,* and *Tapinanthus bangwensis,* the ethyl acetate extracts from the leaves of *Protea ellioti, A. schweinfurthii* and *Vitellaria paradoxa* and the methanol extracts from *Anchomanes difformis* and *Holarrhena floribunda,* showed an additional or synergistic effect in combination with ampicillin only. Those extracts may thus contain potential β-lactamases inhibitors.

On the other hand, 6 extracts were active in combination with ampicillin and also showed a $FICI \leq 1$ when combined with oxacillin. Those are the hexane extract from the roots of *Anchomanes difformis*, the methanol extracts from C*ola gigantea* and *Vitellaria paradoxa* leaves and twigs and the dichloromethane extract from *Vitellaria paradoxa*

leaves. This could suggest that these extracts may counteract resistance mediated by PBP2a production.

In Table 3, we reported available data on phytochemical screenings of all the plants used in this study. Contradictive results were observed but it is well known that the production of secondary metabolites is influenced by numerous factors such as the age of the plant or the leaves, the geographical localization of the plant, the nutrients present in the soil… (Aniszewski 2007; Gershenzon 1984). The influence of such factors on secondary metabolites could be the cause of this discordance, but also the difference of sensitivity of the detection method used. At this stage of the work, it is difficult to predict which class of compounds could be responsible for their direct or indirect antimicrobial activity, each class (alkaloids, anthraquinones, flavonoids, saponins and tannins) containing compounds with antimicrobial activity (Bruneton 2009; Comini et al. 2011; Cowan 1999; Cushnie and Lamb 2005; Karou et al. 2005). Concerning the indirect antimicrobial activity, the synergy between at least one compound of each class and β-lactams has already been studied (Cha et al. 2009; HS Kim et al. 1987; Lee et al. 2010; Shiota et al. 2000; Yu et al. 2005).

As most active extracts were obtained from methanol and could contain tannins (Table 3) which possess antimicrobial activities, we confirmed their presence by TLC. We then compared the antimicrobial activity of these extracts before and after elimination of tannins on a polyamide column. Results are shown in Table 4.

Except for the extracts from the leaves and twigs of *Vitellaria paradoxa*, elimination of tannins totally suppressed both the direct and indirect antimicrobial activities of the methanolic extracts tested. This is coherent with the fact that tannins have already been reported to be bacteriostatic or bactericidal against *Staphylococcus aureus* (Chung et al. 1993).

Moreover, different tannins extracted from plants have been reported as resistance modifying agents. Tellimagrandin I and rugosin B extracted from rose red and corilagin extracted from *Arctostaphylos uva-ursi*, markedly reduced the MIC of β-lactam antibiotics against MRSA (Shimizu et al. 2001; Shiota et al. 2000). Among them, tellimagrandin and corilagin have been reported to be active in combination with oxacillin and revert PBP2a-mediated rather than β-lactamase-mediated resistance. (Santiago et al. 2014; Shiota et al. 2004; Stapleton et al. 2007). The presence of compounds of this type may thus explain the *Cola gigantea* and a part of *Vitellaria paradoxa* effects, which showed a synergistic effect with both oxacillin and ampicillin in the present study.

On the other hand, we may suggest that the methanolic extracts from *Anchomanes difformis, Holarrhena floribunda, Protea elliotii* and *Tapinanthus bangwensis*, which are only active in combination with ampicillin, could contain tannins inhibiting β-lactamases.

This study being the first one to examine the indirect antimicrobial activity of these plants, it is difficult to compare our results with previously published data. We therefore centered our analysis of literature data on direct antimicrobial activity mainly on *Staphylococcus aureus* strains studied by the broth dilution method and cytotoxicity. We also report here already identified compounds and their known antibacterial potential.

Allophylus africanus P. Beauv (Sapindaceae)

Allophylus africanus did not display any antibacterial activity. However, in a study of Sofidiya et al. (2012), the methanolic extract from the leaves showed a direct antimicrobial activity on a strain of *Staphylococcus aureus* with an MIC of 500 mg/L. The difference observed could be explained by the successive extractions made here which could separate plant compounds acting synergistically. Another explanation could be the difference between the bacterial strains used.

Except for pinitol which didn't show any antimicrobial activity, none of identified compounds in this plant were tested for this activity (Manríquez-Torres et al. 2007; Oladosu et al. 2015).

Anchomanes difformis (Blume) Engl. (Araceae)

Different studies showed an antimicrobial activity of methanolic, ethanolic and aqueous extracts from roots of *Anchomanes difformis* by a disc diffusion method on *Staphylococcus aureus* at higher concentrations (Abah et al. 2011; Aliyu et al. 2008). The essential oil of the root part had a MIC of 2000 mg/L on a strain of *Staphylococcus aureus* (Adeleke and Adetunji 2010). Those observations were considered sufficient to justify its use for the treatment of abscesses,

inflammation, intertrigo and conjunctivitis. However, in this study, the root extracts from *Anchomanes difformis* did not show any direct antimicrobial activity at the highest concentration tested (500 mg/L) on the strain MRSA ATCC33591.

Concerning cytotoxicity, none of the extracts tested on the J774 and WI38 showed any cytotoxicity ($IC_{50} > 100$ mg/L). These results are in contradiction with those reported in the study of Bero et al. (2009), which reported a strong cytotoxicity of dichloromethane, methanol and water extracts from the roots of *Anchomanes difformis* (2.2 mg/L $< IC_{50} <$ 26 mg/L). However, in this previous study, the extracts were prepared by maceration at room temperature, while we used here a soxhlet apparatus with a heating at the boiling temperature of each solvent. It could explain the absence of toxicity in our extract as it was already reported that the toxicity level decreases when roots were pre-boiled for a long time (Tchiapke et al. 1980).

Concerning isolated compounds, anchominines A & B, catechin and epicatechin were isolated from the roots of *Anchomanes difformis* (Tchiapke et al. 1980). Anchominines A & B were never tested on bacteria while the MIC of both catechin and epicatechin are higher than 256 mg/L on *Staphylococcus aureus* strains (Stapleton et al. 2004).

Anthocleista schweinfurthii (Gilg.)

No direct antimicrobial activity was detected for all tested root bark extracts of this plant.

This part of plant was never studied for antimicrobial activity while a successive partition of a crude methanolic macerate from the stem bark between water and hexane, dichloromethane, ethyl acetate, and methanol gave four extracts with MICs between 15.625 and 125 mg/L on the strain MRSA ATCC33591 (Ngbolua et al. 2014).

The hexane, dichloromethane and ethyl acetate extracts showed here an additive effect in combination with ampicillin (FICI 0.62–1) with no activity in combination with oxacillin. However, those extracts are quite cytotoxic on the J774 and WI38 cell lines (IC_{50} 23.17 ± 3.36 and 47.10 ± 2.00 respectively), but these activities may not be related.

The root bark was shown to contain schweinfurthiin; bauerenone; bauerenol; 1-hydroxy-3,7,8-trimethoxyxanthone and 1,8-dihydroxy-3,7-dimethoxyxanthone with α-glucosidase inhibitory activity but they were never tested for their antimicrobial activity (Mbouangouere et al. 2007).

Cassia sieberiana DC (Caesalpiniaceae)

No antimicrobial activity was detected for this plant in the present study. However, the traditional use of *Cassia sieberiana* as treatment for abscesses and other infectious diseases was justified based on the fact that concentrations higher than 500 mg/L of leaves extracts prepared by other methods previously showed activity against different bacterial strains (*Bacillus subtilis, Shigella dysenteriae, Escherichia coli, Staphylococcus aureus*) (Asase et al. 2008; Olutayo et al. 2012).

Two polyphenols named l-epicatechol and leucopelargonidol were isolated in the whole plant of *Cassia sieberiana* but their antimicrobial potential is not known (Paris and Etchepare 1967).

Cissampelos mucronata A Rich. (Menispermaceae)

Cissampelos mucronata did not show any direct or indirect antibacterial activity on the strain MRSA ATCC33591. The ethanolic and dichloromethane extracts from the leaves of this plant showed a direct antimicrobial activity against another strain of *Staphylococcus aureus* at much higher concentrations (MIC 3125 and 6250 mg/L respectively) (Nondo et al. 2011).

While different anti-plasmodially active bisbenzylisoquinoleinic alkaloids were identified in the roots, no compounds have already been identified in the leaves or twigs of *Cissampelos mucronata* (Tshibangu et al. 2003).

Cola gigantea A. Chev. Var. gigantea (Sterculiaceae)

The soxhlet ethanol extract from the leaves and twigs of *Cola gigantea* were already tested on a methicillin

Table 3 Published phytochemical screenings

Plants species	Part studied[a]	Extract	Alkaloids	Anthraquinones	Flavonoids	Saponins	Tannins	Reference
Allophylus africanus	AP	Ethanolic maceration	−	+	+	+	+	Oladosu et al. (2013)
Anchomanes difformis	RT	EtOH/MeOH/H_2O 70 :20 :10 maceration	+	nd	+	+	+	Abah et al. (2011)
		Hexane soxhlet	−	−	+	−	−	Doyinsola et al. (2012)
		Ethanolic soxhlet	+	−	−	−	+	Doyinsola et al. (2012)
		Ethanolic maceration	+	−	nd	+	+	Oyetayo (2007)
		Entire plant	−	nd	+	−	+	Aliyu et al. (2008)
Antocleista schweinfurthii	SB	Ethanolic maceration	+	nd	−	−	−	Ngbolua et al. (2014)
		MeOH/DCM 50:50 maceration	−	nd	+	nd	+	(Njayou et al. 2008)
Cassia sieberiana	AP	Ethanolic extract	+	+	+	+	+	(Olutayo et al. 2012)
Cissampelos mucronata	AP	Ethanolic maceration	−	nd	+	+	+	Tanko et al. (2007)
Cola gigantea	TW	MeOH/H2O 4 :1 maceration	+	nd	+	−	−	Onyema and Ajiwe (2014a)
	LF	Ethanolic maceration	+	+	nd	+	+	Sonibare et al. (2009)
Holarrhena floribunda	TW	Methanolic maceration	+	−	−	+	+	Bogne et al. (2007)
	LF	Methanolic maceration on a defatted extract	+	nd	nd	+	+	(Badmus et al. 2009)
		Powder	+	nd	+	nd	+	Nwodo et al. (2007)
Protea elliotii	LF	80 % methanolic maceration	+	−	+	+	+	Raoelison et al. (2013)
Schrankia leptocarpa	AP	Entire plant	+	nd	+	nd	+	Latifou Lagnika (2012)
Spirospermum penduliflorum	AP	Methanolic maceration	+	nd	nd	nd	nd	Udegbunam et al. (2012)
Tapinanthus bangwensis	LF	Methanolic maceration	+	nd	+	+	+	(Ekhaise et al. 2010)
		Methanolic maceration	+	−	+	+	+	Efuntoye et al. (2010)
Vitellaria paradoxa	TW	Ethanolic maceration	+	−	nd	+	+	El − Mahmood et al. (2008)

Table 3 continued

Plants species	Part studied[a]	Extract	Alkaloids	Anthraquinones	Flavonoids	Saponins	Tannins	Reference
		Methanolic maceration	+	−	+	−	+	Ogunwande et al. (2001)
	LF	Methanolic maceration	+	−	−	−	+	(Ogunwande et al., 2001)
		Crude leaves	+	−	−	+	nd	Ndukwe et al. (2007)

+, Present; −, not detected; nd, not determined

sensitive *Staphylococcus aureus* (MSSA) reference strain (ATCC25923) and showed MIC of 250 and 125 mg/L (Agyare et al. 2012). We obtained here a bit higher MIC with our methanolic extract on an MRSA strain (500 mg/L). Recently, Onyema and Ajiwe identified in the chloroformic fraction from crude antimicrobial methanolic extracts of leaves and twigs of *Cola gigantea* several lipophilic compounds possessing antimicrobial properties on different bacterial strains including a strain of *Staphylococcus aureus* (Onyema and Ajiwe 2014a, b). However, these compounds should only be present, probably in low concentration in our lipophilic extracts (hexane or DCM) and not in the most active methanolic extract whose activity is mostly related to the presence of tannins.

Holarrhena floribunda (G. Don) Durand & Schinz (*Apocynaceae)*

Holarrhena floribunda which was shown to be active on different *Bacillus* strains and on *Mycobacterium ulcerans* responsible for the Buruli ulcer (Bogne et al. 2007; Yemoa et al. 2014) appeared to be not directly active at a concentration of 500 mg/L on the strain of MRSA used in this study. The tannins, already evidenced in this plant (Bogne et al. 2007), appeared to be responsible for the indirect antimicrobial activity of the methanol extract in combination with ampicillin. However, this extract is quite cytotoxic with IC_{50} of 16.53 ± 0.43 and 22.81 ± 0.35 mg/L on WI38 and J774 cell lines respectively.

Holarrhena floribunda contains large amount of identified alkaloids (Bogne et al. 2007; Janot et al. 1960; Leboeuf et al. 1969; Nwodo et al. 2007; Paris and Foucaud 1959). Some of them, as berberine, possesses antimicrobial activity on *Staphylococcus aureus* strains (MIC 32–128 mg/L) and shows an additive effect with ampicillin and a synergistic one in combination with oxacillin but targets were not identified (Yu et al. 2005). Some other compounds identified in the plant (conessine, isoquercitroside, progesterone, trichothecolone, and lupeol) were only tested for their direct effect on *Staphylococcus aureus* and for cytotoxicity (Bogne et al. 2007; Chen et al. 2012; Chinworrungsee et al. 2008; Dua et al. 2013; Fotie et al. 2006; Leboeuf et al. 1969; Loukaci et al. 2000; Mathabe et al. 2008; Moriarity et al. 1998; Otsuka et al. 2008; Surup et al. 2014; Yotis and Stanke 1966).

Our results show that isolated antimicrobial compounds seem to be present at low concentration in our extracts and diluted in non active compounds although cytotoxic ones may explain, at least in part, the cytotoxicity observed.

Protea elliotii C. H. Wright (Proeteaceae)

Protea elliotii was mainly studied for its antioxidant properties and its ability to inhibit melanin synthesis (Kamagaju 2014; Tagne et al. 2014). The antimicrobial potential of its aerial parts was explored for the first time in this study. The tested extracts did not show any direct antibacterial activity on the strain MRSA ATCC33591. However, the hexane, ethyl acetate and methanol extracts showed an additive effect in combination with ampicillin (FICI 0.5–1). Tannins were shown to be responsible for the methanol extract effects.

Except for the hexane extract (IC_{50} of 83.16 ± 6.48 and 27.45 ± 1.52 mg/L on the WI38 and J774 cell lines respectively), none of those active extracts appeared to be cytotoxic (IC50 > 100 mg/L).

Table 4 In vitro direct and indirect antibacterial activity on the strain MRSA33591 of the active methanolic extracts before and after polyamide column (n = 3)

Plants species	Part studied	Antibacterial activity against MRSA ATCC33591			
		MIC (mg/L)		FICI AMPI	
		Before	After	Before	After
Anchomanes difformis	AP	>500	>500	0.38–1	1
Cola gigantea	TW	500	>500	0.5–1	1
	LF	500	>500	0.38–1	1
Holarrena floribunda	AP	>500	>500	0.56–1	1
Protea elliotii	AP	>500	>500	0.75–1	1
Tapinanthus bangwensis	AP	500	>500	0.38–1	1
Vitellaria paradoxa	TW	500	>500	0.38–1	0.56–1
	LF	500	>500	0.5–1	0.63–1

No pure compounds were isolated from the aerial parts of *Protea elliotii*. Three compounds were isolated from the root bark of this plant : oleic acid, β-sitosterol and 2-tridecanone (Kamagaju Léocadie 2014). The MIC of oleic acid is 1000 mg/L and that of β-sitosterol is 25 mg/mL on *Staphylococcus aureus* (Dilika et al. 2000; Odiba et al. 2014).

Shrankia leptocarpa DC. (Mimosaceae)

Different extracts from *Schrankia leptocarpa* are reported to have MICs comprised between 0.625 and 2.5 mg/L on a methicillin sensitive *Staphylococcus aureus.* The diethylether extract was the most active one (Latifou Lagnika 2012). Those results are in accordance with the activity we found with the hexane extract, in combination with ampicillin (FICI 0.62–1). Further fractionation of this extract would be of interest because it did not show any cytotoxicity ($IC_{50} > 100$ mg/L on both cell lines used here).

By a trypanocidal bioassay-guided fractionation of aerial parts of *S. leptocarpa*, different compounds were isolated, namely 1,7-bis-(4-hydroxyphenyl)-3-hepten-5-one; 5-hydroxy-1,7-bis(4-hydroxyphenyl)heptan-3-one; methyl-1-chlorogenate; éthyl-1-chlorogenate; caffeic acid; *p*-coumarylagmatine; manghasline; mauritianin; quercetin 3-neohesperidoside (Lagnika et al. 2012). Amongst them, only caffeic acid was tested for his antimicrobial activity and showed a MIC higher than 50 mg/L on *Staphylococcus aureus* (Fu et al. 2010).

Spirospermum penduliflorum Thouars (Menispermaceae)

Spirospermum penduliflorum was mainly studied for its antiplasmodial alkaloids content but also for its vasorelaxant activity (Raoelison et al. 2013; Ratsimamanga-Urverg et al. 1992). However, this plant was never studied for its antimicrobial activity. We detected here that the dichloromethane extract from the aerial parts of this plant presented an additive effect in combination with ampicillin (FICI 0.56–1). However, this extract showed a quite high cytotoxicity with IC_{50} of 10.10 ± 0.20 and 4.82 ± 0.67 mg/L on the WI38 and J774 cell lines. Further studies are needed to determine whether indirect antimicrobial activity and cytotoxicity are due to the same compounds but aporphine alkaloids could be responsible for the observed cytotoxicity (Stévigny et al. 2002).

Two vasorelaxant aporphine alkaloids were isolated from the leaves of *S. penduliflorum* namely dicentrine and néolitsine (Raoelison et al. 2013). Those two compounds are antihelmintic and a dicentrine derivative (dicentrine methane) has a MIC comprised between 50 and 300 mg/L on *Staphylococcus aureus* strains.

Tapinanthus bangwensis (Engl. & Krause) Danser (Loranthaceae)

The dichloromethane extract from the aerial parts showed an additive effect in combination with ampicillin. However, this extract is cytotoxic at much more

lower concentrations with IC_{50} of 28.89 ± 1.98 and 29.63 ± 8.64 mg/L on WI38 and J774 cell lines respectively.

We showed that tannins were responsible for the direct and indirect antimicrobial activity of the methanolic extract which did not show any cytotoxicity on both cell lines used in this study (IC_{50} > 100 - mg/L on WI38 and J774).

Gallic acid derivatives were identified in the plant, among which methyl syringate possessing a significant antibacterial activity against *Staphylococcus aureus* (Patrick-Iwuanyanwu et al. 2014; Russell et al. 1990). Shibata et al. (2005) also demonstrated that alkyl gallates intensified beta-lactam susceptibility in methicillin-resistant *Staphylococcus aureus*. Catechin, epicatechin and derivatives were also identified. While the MIC of catechin and epicatechin are higher than 256 mg/L, it is known that epicatechin 3-*O*-gallate has a MIC comprised between 64 and 128 mg/L on *Staphylococcus aureus* strains (Agbo et al. 2013; Ogechukwu et al. 2012; Stapleton et al. 2004). Moreover, catechin derivatives may possess, as epicatechin-3-*O*-gallate, the capacity to modulate β-lactam resistance in MRSA strains (Stapleton et al. 2004; Shiota et al. 1999). Other compounds were also identified but were not tested for their antimicrobial properties (Agbo et al. 2013; Ogechukwu et al. 2011; Omeje et al. 2011).

Vitellaria paradoxa C.F. Gaertn. ssp. *paradoxa* (Sapotaceae)

Vitellaria paradoxa is an immensely popular tree because the fat extract from the kernels called shea butter is widely used in cosmetic industries (Hall et al. 1996). The antimicrobial activity of its stem bark and leaves was already studied on strains of *Staphylococcus aureus* but with an agar well diffusion method at high concentration (50 g/L) (Ayankunle et al. 2012; Ogunwande et al. 2001).

The dichloromethane extract from the leaves showed the best direct and indirect antimicrobial activities. However, this extract was also cytotoxic with an IC_{50} of 7.17 ± 2.93 and 27.56 ± 0.13 mg/L on the WI38 and J774 cell lines respectively. In fact, it has already been described that the prolonged administration of the plant extract at high doses may adversely affect the functions of some vital organs, but compounds responsible for both effects may be different (Ayankunle et al. 2012).

Tannins already identified in this plant (Ndukwe et al. 2007; El-Mahmood et al. 2008) were shown to be only responsible for a part of the indirect antimicrobial activity of both twigs and leaves methanol extracts. However, it is the only plant which showed a residual activity after the polyamide separation.

Though many studies focused on shea butter and kernels, only a few identified compounds from the root bark of *Vitellaria paradoxa* among which two antibacterial compounds named 2-*O*-butyl-1-*O*-(2′-ethylhexyl) benzene-1,8-dicarboxylate and 1-phenyl-1,4-pentanedione (Garba and Salihu 2011; Tapondjou et al. 2011). However leaves and twigs were not much studied and no compounds were isolated from them.

Conclusion

In our study, we tested for the first time the direct and indirect antimicrobial activity of 12 plants used in traditional medicine in Africa on a methicillin resistant *Staphylococcus aureus* strain. Several extracts showed direct antimicrobial activity or seem to reverse the resistance of MRSA to β-lactams. Though reported activities may be considered as moderate, they may contain low concentrations of interesting compounds and this may explain at least to some extend their use in traditional medicine. Nevertheless, some extracts showed cytotoxicity which may be related to different compounds than those responsible for the antimicrobial effects, but needs further research to determine their safety in traditional use.

We also showed that tannins were responsible for most antimicrobial activities of methanolic extracts (*Anchomanes difformis, Cola gigantea, Holarrhena floribunda, P. eliotii, Tapinanthus bangwensis* and *Vitellaria paradoxa*). The last one is the only methanolic extract presenting a residual activity after removal of tannins on a polyamide column.

The best direct and indirect antimicrobial activity was obtained with the dichloromethane extract from the leaves of *Vitellaria paradoxa* which presented a MIC of 250 mg/L on MRSA33591 and FICI ranges of 0.14–1 and 0.51–1 in combination with ampicillin and oxacillin respectively. Further research is needed in order to identify active molecules and also determine those responsible for the high cytotoxicity of this extract. The hexane extract from the roots of *Anchomanes difformis* also shows interesting properties,

with FICI ranges of 0.18–1 and 0.60–2 in combination with ampicillin and oxacillin respectively, warranting additional studies to identify the active compounds therein.

We also showed here that only a few pure antimicrobial compounds were isolated from the plants studied. It will be thus really interesting to fractionate active extracts.

We have however to acknowledge some limitations of this study, related to the fact that that we studied a single bacterial strain and looked for synergy with two β-lactams only. This design was adopted in order to obtain a first indication about the potential mechanisms of the synergisms observed. In spite of this, and pending for further studies aiming at identifying the active compounds and extending our observation to other bacterial strains or drugs, this work opens the doors to the identification of new resistance modifying agents active on the β-lactams resistance mechanisms from methicillin resistant *Staphylococcus aureus*.

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References

Abah SE, Egwari L, Mosaku TO (2011) In vitro antimicrobial screening on *Anchomanes difformis* (Blume) Engl. leaves and rhizomes against selected pathogens of public health importance. Adv Biol Res 5:221–225

Abreu AC, McBain AJ, Simões M (2012) Plants as sources of new antimicrobials and resistance-modifying agents. Nat Prod Rep 29:1007–1021. doi:10.1039/C2NP20035J

Adeleke O, Adetunji T (2010) Antimicrobial activity of *Anchomanes difformis* (Blume) Engl. [family ARACEAE]. J Life Phys Sci 2:87–90

Adjanohoun EJ, Adjakidje V, Ahyi MR, Aké assi L, Akoegninou A, d'Almeida J, Apovo F, Boukef K, Chadare M, Cusset G, Dramane K, Eyme J, Gassita JN, Gbaguidi N, Goudote E, Guinko P, Houngnon P, Lo I, Keita A, Kiniffo HV, Kone-Bamba D, Musampa Nseyya A, Saadou M, Sodogandji T, de Souza S, Tchabi A, Zinsou T (1989) Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. In: Médecine Traditionnelle et Pharmacopée. Cotonou

Agbo MO, Lai D, Okoye FBC, Osadebe PO, Proksch P (2013) Antioxidative polyphenols from Nigerian mistletoe *Loranthus micranthus* (Linn.) parasitizing on Hevea brasiliensis. Fitoterapia 86:78–83. doi:10.1016/j.fitote.2013.02.006

Agyare C, Koffuor GA, Boamah VE, Adu F, Mensah KB, Adu-Amoah L (2012) Antimicrobial and anti-inflammatory activities of *Pterygota macrocarpa* and *Cola gigantea* (Sterculiaceae). Evid Based Complement Altern Med 2012:e902394. doi:10.1155/2012/902394

Ahua KM, Ioset J-R, Diallo D, Mauël J, Hostettmann K (2007) Antileishmanial activities associated with plants used in the Malian traditional medicine. J Ethnopharmacol 110:99–104

Aliyu AB, Musa AM, Oshanimi JA, Ibrahim HA, Oyewale AO (2008) Phytochemical analyses and mineral elements composition of some medicinal plants of Northern Nigeria. Niger J Pharm Sci 7:119–125

Aniszewski T (2007) Alkaloids: secrets of life: aklaloid chemistry, biological significance, applications and ecological role. Elsevier, Amsterdam

Asase A, Kokubun T, Grayer RJ, Kite G, Simmonds MSJ, Oteng-Yeboah AA, Odamtten GT (2008) Chemical constituents and antimicrobial activity of medicinal plants from Ghana: *Cassia sieberiana*, *Haematostaphis barteri*, *Mitragyna inermis* and *Pseudocedrela kotschyi*. Phytother Res 22:1013–1016. doi:10.1002/ptr.2392

Ayankunle AA, Kolawole OT, Adesokan AA, Akiibinu MO (2012) Antibacterial activity and sub-chronic toxicity studies of vitellaria paradoxa stem bark extract. J Pharmacol Toxicol 7:298–304. doi:10.3923/jpt.2012.298.304

Badmus JA, Odunola OA, Obuotor EM, Oyedapo OO (2009) Phytochemicals and in vitro antioxidant potentials of defatted methanolic extract of *Holarrhena floribunda* leaves. Afr J Biotechnol 9:340–346

Balogun SO, Oladosu IA, Liu Z (2014) Chemical compositions and antioxidant potential of essential oils from leaves and flowers of *Allophylus africanus*. J Essent Oil Bear Plants 17:769–775. doi:10.1080/0972060X.2014.895176

Barber M, Waterworth PM (1964) Penicillinase-resistant Penicillins. Br Med J 2:344–349

Bero J, Ganfon H, Jonville M-C, Frédérich M, Gbaguidi F, DeMol P, Moudachirou M, Quetin-Leclercq J (2009) In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J Ethnopharmacol 122:439–444. doi:10.1016/j.jep.2009.02.004

Bogne KP, Penlap BV, Lontsi D, Etoa F-X (2007) Antibacterial activities of the extracts and conessine from Holarrhena floribunda G. Don. (Apocynaceae). Afr J Tradit Complement Altern Med 4:352–356

Bonapace CR, Bosso JA, Friedrich LV, White RL (2002) Comparison of methods of interpretation of checkerboard synergy testing. Diagn Microbiol Infect Dis 44:363–366. doi:10.1016/S0732-8893(02)00473-X

Bruneton J (2009) Pharmacognosie, phytochimie, plantes médicinales (4e ed.), Tec & Doc ed Lavoisier, Paris

Buyck JM, Tulkens PM, Van Bambeke F (2015) Activities of antibiotic combinations against resistant strains of Pseudomonas aeruginosa in a model of infected THP-1 monocytes. Antimicrob Agents Chemother 59:258–268. doi:10.1128/AAC.04011-14

Cha J-D, Moon S-E, Kim J-Y, Jung E-K, Lee Y-S (2009) Antibacterial activity of sophoraflavanone G isolated from

the roots of *Sophora flavescens* against methicillin-resistant *Staphylococcus aureus*. Phytother Res 23:1326–1331. doi:10.1002/ptr.2540

Chanda S, Rakholiya K (2011) Indian combination therapy: synergism between natural plant extracts and antibiotics against infectious diseases. In: Mendez-Vilas A (ed) Science against microbial pathogens: communicating current research and technological advances. Formatex Research Center, Spain, pp 520–529

Chen H-Y, Ye X-L, Cui X-L, He K, Jin Y-N, Chen Z, Li X-G (2012) Cytotoxicity and antihyperglycemic effect of minor constituents from rhizoma coptis in HepG2 cells. Fitoterapia 83:67–73. doi:10.1016/j.fitote.2011.09.014

Chinworrungsee M, Wiyakrutta S, Sriubolmas N, Chuailua P, Suksamrarn A (2008) Cytotoxic activities of trichothecenes isolated from an endophytic fungus belonging to order Hypocreales. Arch Pharm Res 31:611–616. doi:10.1007/s12272-001-1201-x

Chung K-T, Stevens SE Jr, Lin W-F, Wei CI (1993) Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. Lett Appl Microbiol 17:29–32. doi:10.1111/j.1472-765X.1993.tb01428.x

Coates A, Hu Y, Bax R, Page C (2002) The future challenges facing the development of new antimicrobial drugs. Nat Rev Drug Discov 1:895–910. doi:10.1038/nrd940

Comini LR, Núñez Montoya SC, Páez PL, Argüello GA, Albesa I, Cabrera JL (2011) Antibacterial activity of anthraquinone derivatives from *Heterophyllaea pustulata* (Rubiaceae). J Photochem Photobiol, B 102:108–114. doi:10.1016/j.jphotobiol.2010.09.009

Cowan MM (1999) Plant Products as Antimicrobial Agents. Clin Microbiol Rev 12:564–582

Cushnie TPT, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26:343–356. doi:10.1016/j.ijantimicag.2005.09.002

Dilika F, Bremner PD, Meyer JJM (2000) Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia 71:450–452. doi:10.1016/S0367-326X(00)00150-7

Doyinsola Idowu, Adedayo Adebiyi, Olutayo Olajide, Michael Afolayan, Abayomi Orishadipe, Moses Omojola, Thomas Sunday (2012) Phytochemical, antioxidant and cytotoxicity properties of *Anchomanes difformis* (Bl.) Engl. tuber extract. Int J Appl Chem 8:173–181

Drawz SM, Bonomo RA (2010) Three decades of beta-lactamase inhibitors. Clin Microbiol Rev 23:160–201. doi:10.1128/CMR.00037-09

Dua VK, Verma G, Singh B, Rajan A, Bagai U, Agarwal DD, Gupta NC, Kumar S, Rastogi A (2013) Anti-malarial property of steroidal alkaloid conessine isolated from the bark of *Holarrhena antidysenterica*. Malar. J. 12:194. doi:10.1186/1475-2875-12-194

Efuntoye MO, Ayodele AE, Thomas BT, Ajayi TO (2010) Does host plant affect the antibacterial activity of *Tapinanthus bangwensis* (Engl. and K. Krause) Danser (Loranthaceae). J Med Plants Res 4:1281–1284

Ekhaise FO, Ofoezie VG, Enobakhare DA (2010) Antibacterial properties and preliminary phytochemical analysis of methanolic extract of mistletoe (*Tapinanthus bangwensis*). Bayero J Pure Appl Sci 3:65–68. doi:10.4314/bajopas.v3i2.63223

Eliopoulos G, Moellering RC (1996) Antimicrobial combinations. Antibiotics in laboratory medicine. Lorian V, Baltimore, pp 330–396

El-Mahmood AM, Doughari JH, Ladan N (2008) Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms. Afr J Pharm Pharmacol 2:089–094

EUCAST (2000) Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. Clin Microbiol Infect 6:503–508. doi:10.1046/j.1469-0691.2000.00149.x

Fotie J, Bohle DS, Leimanis ML, Georges E, Rukunga G, Nkengfack AE (2006) Lupeol long-chain fatty acid esters with antimalarial activity from *Holarrhena floribunda*. J Nat Prod 69:62–67. doi:10.1021/np050315y

Fu J, Cheng K, Zhang Z, Fang R, Zhu H (2010) Synthesis, structure and structure–activity relationship analysis of caffeic acid amides as potential antimicrobials. Eur J Med Chem 45:2638–2643. doi:10.1016/j.ejmech.2010.01.066

Garba S, Salihu L (2011) Antibacterial activities of 2-*O*-butyl-1-*O*-(2′-ethylhexyl) benzene-1,8-dicarboxylate and 1-phenyl-1,4-pentanedione isolated from *Vitellaria paradoxa* root bark. Asian J Sci Res 4:149–157. doi:10.3923/ajsr.2011.149.157

Gershenzon J (1984) Changes in the levels of plant secondary metabolites under water and nutrient stress. In: Timmermann BN, Steelink C, Loewus FA (eds) Phytochemical adaptations to stress, recent advances in phytochemistry. Springer, New York, pp 273–320

Gibbons S (2004) Anti-staphylococcal plant natural products. Nat Prod Rep 21:263–277. doi:10.1039/B212695H

Gibbons S (2005) Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochem Rev 4:63–78. doi:10.1007/s11101-005-2494-9

Hadler JL, Petit S, Mandour M, Cartter M (2012) Trends in Invasive Infection with Methicillin-Resistant Staphylococcus aureus, Connecticut, USA, 2001–2010. Emerg. Infect. Dis. J. 18:917–924

Hall JB, Aebischer DP, Tomlinson HF, Osei-Amaning E, Hindle JR (1996) *Vitellaria paradoxa*: a monograph. School of Agricultural and Forest Sciences, University of Wales

Hemaiswarya S, Kruthiventi AK, Doble M (2008) Synergism between natural products and antibiotics against infectious diseases. Phytomedicine 15:639–652. doi:10.1016/j.phymed.2008.06.008

Houghton PJ, Raman A (1998) Laboratory handbook for the fractionation of natural extracts. Chapman and Hall, London

Janot MM, Cave A, Goutarel R (1960) Steroid alkaloids. Holaphyllamine and holamine, alkaloids from *Holarrhena floribunda* (G. Don) Dur. and Schinz. Comptes Rendus Hebd. Séances Académie Sci. 251:559–561

Jiofack T, Fokunang C, Guedje N, Kemeuze V, Fongnzossie E, Nkongmeneck BA, Mapongmetsem PM, Tsabang N (2010) Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. Int J Med Med Sci 2:60–79

Johnson AP (2011) Methicillin-resistant *Staphylococcus aureus*: the European landscape. J Antimicrob Chemother 66:43–48. doi:10.1093/jac/dkr076

Kalant H (1965) The pharmacology of semisynthetic antibiotics. Can Med Assoc J 93:839–843

Kamagaju L (2014) Problem of skin depigmentation in Rwanda. Modulators of tyrosinase extracted from plants used in traditional medicine. Thesis in Biomedical and Pharmaceutical Sciences, ULB, Brussels

Karou D, Savadogo A, Canini A, Yameogo S, Montesano C, Simpore J, Colizzi V, Traore AS (2005) Antibacterial activity of alkaloids from Sida acuta. Afr J Biotechnol. doi:10.4314/ajb.v4i12.71463

Kim HS, Han SS, Oh KW, Jeong TS, Nam KY (1987) Effects of Ginseng saponin on the antimicrobial activities of some antibiotics. Korean J Mycol 15:87–91

Kuete V (2010) Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med 76:1479–1491. doi:10.1055/s-0030-1250027

Lagnika L, Weniger B, Attioua B, Jensen O, Anthaume C, Sanni A, Kaiser M, Lobstein A, Vonthron-Senecheau C (2012) Trypanocidal activity of diarylheptanoids from *Schrankia leptocarpa* DC. South Afr J Bot 83:92–97. doi:10.1016/j.sajb.2012.06.011

Latifou Lagnika UP (2012) Chemical analysis, antimicrobial and antioxidant activities of eight extracts from *Schrankia leptocarpa* L. Afr J Biotechnol 11:13739–13745. doi:10.5897/AJB12.633

Leboeuf M, Cavé A, Goutarel R (1969) Steroid alkaloids. LXXXII. (16). Chemical composition of the leaves of *Holarrhena floribunda* Dur. et Schinz. Isolation of progesterone and 4 new alkaloids: methylholaphylline, holaphyllinol, holaphyllidine and dihydroholaphyllamine. Ann Pharm Fr 27:217–228

Lee Y-S, Kang O-H, Choi J-G, Oh Y-C, Keum J-H, Kim S-B, Jeong G-S, Kim Y-C, Shin D-W, Kwon D-Y (2010) Synergistic effect of emodin in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. Pharm Biol 48:1285–1290. doi:10.3109/13880201003770150

Loukaci A, Kayser O, Bindseil K, Siems K, Frevert J, Abreu PM (2000) New trichothecenes isolated from *Holarrhena floribunda*. J Nat Prod 63:52–56

Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339:520–532. doi:10.1056/NEJM199808203390806

Manríquez-Torres J, Zúñiga-Estrada A, González-Ledesma M, Torres-Valencia JM (2007) The antibacterial metabolites and proacacipetalin from *Acacia cochliacantha*. J Mex Chem Soc 51:228–231

Mathabe MC, Hussein AA, Nikolova RV, Basson AE, Meyer JJM, Lall N (2008) Antibacterial activities and cytotoxicity of terpenoids isolated from *Spirostachys africana*. J Ethnopharmacol 116:194–197. doi:10.1016/j.jep.2007.11.017

Mbouangouere RN, Tane P, Ngamga D, Khan SN, Choudhary MI, Ngadjui BT (2007) A new steroid and α-glucosidase inhibitors from *Anthocleista schweinfurthii*. Res J Med Plant 1:106–111

Mezui C, Longo F, Nkenfou C, Sando Z, Ndeme E, Vernyuy Tan P (2015) Evalutation of acute and subacute tovicity of stem bark aqueous extract of *Anthocleista schweinfurthii* (Loganiaceae). World J Pharm Pharm Sci 4:197–208

Miller RR, Price JR, Batty EM, Didelot X, Wyllie D, Golubchik T, Crook DW, Paul J, Peto TEA, Wilson DJ, Cule M, Ip CLC, Day NPJ, Moore CE, Bowden R, Llewelyn MJ (2014) Healthcare-associated outbreak of meticillin-resistant *Staphylococcus aureus* bacteraemia: role of a cryptic variant of an epidemic clone. J Hosp Infect 86:83–89. doi:10.1016/j.jhin.2013.11.007

Moriarity DM, Huang J, Yancey CA, Zhang P, Setzer WN, Lawton RO, Bates RB, Caldera S (1998) Lupeol is the cytotoxic principle in the leaf extract of *Dendropanax* cf. *querceti*. Planta Med 64:370–372

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Ndukwe IG, Amupitan JO, Isah Y, Adegoke KS (2007) Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa* (Gaertn. F). Afr J Biotechnol 6:1905–1909

Ngbolua KN, Mubindoukila Rosie Esther N, Mpiana Pius T, Ashande Masengo C, Robijaona Baholy, Ruphin Fatiany Pierre, Takoy L, Ekutsu Grégoire E, Gbolo Zoawe B (2014) In vitro assessment of antibacterial and antioxidant activities of a congolese medicinal plant species *Anthocleista schweinfurthii* Gilg (Gentianaceae). J Mod Drug Discov Drug Deliv Res 3:1–6. doi:10.15297/JMDDR.V1I3.03

Njayou FN, Moundipa PF, Tchana AN, Ngadjui BT, Tchouanguep FM (2008) Inhibition of microsomal lipid peroxidation and protein oxidation by extracts from plants used in Bamun folk medicine (Cameroon) against hepatitis. Afr J Tradit Complement Altern Med 5:278–289

Nondo RSO, Mbwambo ZH, Kidukuli AW, Innocent EM, Mihale MJ, Erasto P, Moshi MJ (2011) Larvicidal, antimicrobial and brine shrimp activities of extracts from *Cissampelos mucronata* and *Tephrosia villosa* from coast region, Tanzania. BMC Complement Altern Med 11:33. doi:10.1186/1472-6882-11-33

Nwodo NJ, Brun R, Osadebe PO (2007) In vitro and in vivo evaluation of the antitrypanosomal activity of fractions of *Holarrhena africana*. J Ethnopharmacol 113:556–559. doi:10.1016/j.jep.2007.07.018

Odiba JO, Musa AM, Hassan HS, Yahaya SM, Okolo EI (2014) Antimicrobial activity of isolated Stigmast-5-en-3β-ol (β-sitosterol) from honeybee propolis from North-Western, Nigeria. Int J Pharma Sci Res 5:908–918

Ogechukwu OE, Ogoamaka OP, Sylvester NC, Hassan A, Debbab A, Okechukwu EC, Kawamura A, Peter P (2011) Steroids and triterpenoids from Eastern Nigeria mistletoe, *Loranthus micranthus* Linn. (Loranthaceae) parasitic on *Kola acuminata* with immunomodulatory potentials. Phytochem Lett 4:357–362. doi:10.1016/j.phytol.2011.07.011

Ogechukwu OE, Ogoamaka OP, Kawamura A, Hassan A, Debbab A et al (2012) Three-(−) catechin-*O*-rhamnosides from the Eastern Nigeria Mistletoe with potent immunostimulatory and antioxidant activities. Biomolecul 1:2

Ogunwande IA, Bello MO, Olawore ON, Muili KA (2001) Phytochemical and antimicrobial studies on *Butyrospermum paradoxum*. Fitoterapia 72:54–56. doi:10.1016/S0367-326X(00)00238-0

Oladosu IA, Balogun SO, Ademowo GO (2013) Phytochemical screening, antimalarial and histopathological studies of *Allophylus africanus* and *Tragia benthamii*. Chin. J. Nat. Med. 11:371–376. doi:10.1016/S1875-5364(13)60054-0

Oladosu IA, Balogun SO, Liu Z-Q (2015) Chemical constituents of Allophylus africanus. Chin. J. Nat. Med. 13:133–141. doi:10.1016/S1875-5364(15)60017-6

Olutayo Olajide, Michael Afolayan, John Adewusi Adepoju, Olusola Adeyanju (2012) Antimicrobial activity and Elemental analysis of Cassia siberiana leaves Using Atomic Absorption Spectrometer. J. Nat. Prod. Plant Resour. 2:9–18

Omeje EO, Osadebe PO, Nworu CS, Nwodo JN, Obonga WO, Kawamura A, Esimone CO, Proksch P (2011) A novel sesquiterpene acid and an alkaloid from leaves of the Eastern Nigeria mistletoe, *Loranthus micranthus* with potent immunostimulatory activity on C57BL6 mice splenocytes and CD69 molecule. Pharm Biol 49:1271–1276. doi:10.3109/13880209.2011.621129

Onyema CT, Ajiwe VIE (2014a) Phytochemical and anti-microbial analysis of the leaves of *Cola gigantea* (Sterculiaceae). Int J Appl Sci Technol 4:119–126

Onyema CT, Ajiwe VIE (2014b) Phytochemical and antimicrobial analysis of the stems of *Cola gigantea* (Sterculiacea). Int J Eng Sci 3:01–11

Otsuka N, Liu M-H, Shiota S, Ogawa W, Kuroda T, Hatano T, Tsuchiya T (2008) Anti-methicillin resistant *Staphylococcus aureus* (MRSA) compounds isolated from *Laurus nobilis*. Biol Pharm Bull 31:1794–1797

Oyetayo VO (2007) Comparative studies of the phytochemical and antimicrobial properties of the leaf, stem and tuber of *Anchomanes difformis*. J Pharmacol Toxicol 2:407–410. doi:10.3923/jpt.2007.407.410

Paris R, Etchepare S (1967) On the polyphenols of *Cassia sieberiana* DC. Isolation of l-epicatechol and leucopelargonidol. Ann Pharm Fr 25:343–346

Paris RR, Foucaud A (1959) Flavonoids in the leaves of *Holarrhena floribunda* G. Don (Durand and Schinz): isolation of a flavonoside identified as isoquercitroside. Comptes Rendus Hebd Séances Académie Sci 248:2634–2635

Patrick-Iwuanyanwu KC, Onyeike EN, Adhikari A (2014) Isolation, identification and characterization of gallic acid derivatives from leaves of *Tapinanthus bangwensis*. J Nat Prod 7:14–19

Raoelison EG, Rafamantanana MH, Razafindrazaka R, Randriantsoa A, Urverg-Ratsimamanga S, Morel N, Quetin-Leclercq J (2013) Vasorelaxant alkaloids from *Spirospermum penduliflorum* (Menispermaceae), a plant used to treat hypertension in malagasy traditional medicine. Nat Prod Commun Int J Commun Rev 8:575–578

Ratsimamanga-Urverg S, Rasoanaivo P, Ramiaramanana L, Milijaona R, Rafatro H, Verdier F, Rakoto-Ratsimamanga A, Le Bras J (1992) In vitro antimalarial activity and chloroquine potentiating action of two bisbenzylisoquinoline enantiomer alkaloids isolated from *Strychnopsis thouarsii* and *Spirospermum penduliflorum*. Planta Med 58:540–543. doi:10.1055/s-2006-961545

Rodvold KA, McConeghy KW (2014) Methicillin-resistant *Staphylococcus aureus* therapy: past, present, and future. Clin Infect Dis 58:S20–S27. doi:10.1093/cid/cit614

Russell KM, Molan PC, Wilkins AL, Holland PT (1990) Identification of some antibacterial constituents of New Zealand manuka honey. J Agric Food Chem 38:10–13. doi:10.1021/jf00091a002

Sandora TJ, Goldmann DA (2012) Preventing lethal hospital outbreaks of antibiotic-resistant bacteria. N Engl J Med 367:2168–2170. doi:10.1056/NEJMp1212370

Santiago C, Pang EL, Lim K-H, Loh H-S, Ting KN (2014) Reversal of ampicillin resistance in MRSA via inhibition of penicillin-binding protein 2a by *Acalypha wilkesiana*. BioMed Res Int. doi:10.1155/2014/965348

Sarker SD, Nahar L, Kumarasamy Y (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods Nat Prod Res 42:321–324. doi:10.1016/j.ymeth.2007.01.006

Schmelzer GH, Gurib-Fakim A (2008) Ressources végétales de l'Afrique tropicale 11 (1): Plantes médicinales. Schmelzer G.H & Gurib-Fakim A, Pay-Bas

Shibata H, Kondo K, Katsuyama R, Kawazoe K, Sato Y, Murakami K, Takaishi Y, Arakaki N, Higuti T (2005) Alkyl gallates, intensifiers of β-lactam susceptibility in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 49:549–555. doi:10.1128/AAC.49.2.549-555.2005

Shimizu M, Shiota S, Mizushima T, Ito H, Hatano T, Yoshida T, Tsuchiya T (2001) Marked potentiation of activity of β-lactams against methicillin-resistant *Staphylococcus aureus* by corilagin. Antimicrob Agents Chemother 45:3198–3201. doi:10.1128/AAC.45.11.3198-3201.2001

Shiota S, Shimizu M, Mizushima T, Ito H, Hatano T, Yoshida T, Tsuchiya T (1999) Marked reduction in the minimum inhibitory concentration (MIC) of beta-lactams in methicillin-resistant *Staphylococcus aureus* produced by epicatechin gallate, an ingredient of green tea (*Camellia sinensis*). Biol Pharm Bull 22:1388–1390

Shiota S, Shimizu M, Mizusima T, Ito H, Hatano T, Yoshida T, Tsuchiya T (2000) Restoration of effectiveness of β-lactams on methicillin-resistant *Staphylococcus aureus* by tellimagrandin I from rose red. FEMS Microbiol Lett 185:135–138. doi:10.1111/j.1574-6968.2000.tb09051.x

Shiota S, Shimizu M, Sugiyama J, Morita Y, Mizushima T, Tsuchiya T (2004) Mechanisms of action of corilagin and tellimagrandin I that remarkably potentiate the activity of beta-lactams against methicillin-resistant *Staphylococcus aureus*. Microbiol Immunol 48:67–73

Sibanda T, Okoh AI (2007) The challenges of overcoming antibiotic resistance: plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr J Biotechnol. doi:10.4314/ajb.v6i25.58241

Sofidiya MO, Jimoh Florence O, Aliero Adamu A, Afolayan Anthony J, Odukoya Olukemi A, Familoni Oluwole B (2012) Evaluation of antioxidant and antibacterial properties of six Sapindaceae members. J Med Plants Res. doi:10.5897/JMPR11.1364

Sonibare M, Soladoye M, Esan O, Sonibare O (2009) Phytochemical and antimicrobial studies of four species of Cola Schott & Endl. (Sterculiaceae). Afr J Tradit Complement Altern Med 6:518–525

Stapleton PD, Shah S, Anderson JC, Hara Y, Hamilton-Miller JMT, Taylor PW (2004) Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates. Int J Antimicrob Agents 23:462–467. doi:10.1016/j.ijantimicag.2003.09.027

Stapleton PD, Shah S, Ehlert K, Hara Y, Taylor PW (2007) The β-lactam-resistance modifier (–)-epicatechin gallate alters the architecture of the cell wall of *Staphylococcus aureus*. Microbiol Read Engl 153:2093–2103. doi:10.1099/mic.0.2007/007807-0

Stevigny C, Block S, De Pauw-Gillet MC, de Hoffmann E, Llabrès G, Adjakidjé V, Quetin-Leclercq J (2002) Cytotoxic aporphine alkaloids from *Cassytha filiformis*. Planta Med 68:1042–1044. doi:10.1055/s-2002-35651

Stévigny C, Block S, De Pauw-Gillet MC, de Hoffmann E, Llabrès G, Adjakidjé V, Quetin-Leclercq J (2002) Cytotoxic aporphine alkaloids from *Cassytha filiformis*. Planta Med 68:1042–1044. doi:10.1055/s-2002-35651

Surup F, Medjedović A, Szczygielski M, Schroers H-J, Stadler M (2014) Production of trichothecenes by the apple sooty blotch fungus *Microcyclospora tardicrescens*. J Agric Food Chem 62:3525–3530. doi:10.1021/jf500153d

Tagne RS, Telefo BP, Nyemb JN, Yemele DM, Njina SN, Goka SMC, Lienou LL, Nwabo Kamdje AH, Moundipa PF, Farooq AD (2014) Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. Asian Pac J Trop Med 7(S1):S442–S447. doi:10.1016/S1995-7645(14)60272-8

Tanko Y, Yaro AH, Isa AI, Yerima M, Saleh MIA, Mohammed A (2007) Toxicological and hypoglycemic studies on the leaves of *Cissampelos mucronata* (Menispermaceae) on blood glucose levels of streptozocin-induced diabetic wistar rats. J Med Plants Res 1:113–116

Tapondjou LA, Nyaa LBT, Tane P, Ricciutelli M, Quassinti L, Bramucci M, Lupidi G, Ponou BK, Barboni L (2011) Cytotoxic and antioxidant triterpene saponins from *Butyrospermum parkii* (Sapotaceae). Carbohydr Res 346:2699–2704. doi:10.1016/j.carres.2011.09.014

Taylor PW (2013) Alternative natural sources for a new generation of antibacterial agents. Int J Antimicrob Agents 42:195–201. doi:10.1016/j.ijantimicag.2013.05.004

Tchiapke L, Balansard G, Bernard P, Placidi M (1980) Chemical and toxicological study of *Anchomanes difformis* (Engl). Herba Hung 19:55–63

Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. Am J Med 119:S3–S10. doi:10.1016/j.amjmed.2006.03.011

Tshibangu JN, Wright AD, König GM (2003) HPLC isolation of the anti-plasmodially active bisbenzylisoquinone alkaloids present in roots of *Cissampelos mucronata*. Phytochem Anal 14:13–22. doi:10.1002/pca.673

Udegbunam SO, Udegbunam RI, Okpo OS, Muogbo CJ (2012) Evaluation of the wound healing potential of *Protea madiensis* olive leaf extract in rats. Afr J Biotechnol 11:15719–15727. doi:10.5897/AJB12.517

Wagner H, Bladt S (1996) Plant drug analysis: a thin layer chromatography atlas. Springer, Berlin

Yemoa A, Gbenou J, Affolabi D, Moudachirou M, Bigot A, Anagonou S, Portaels F, Martin A, Quetin-Leclercq J (2014) Beninese medicinal plants as a source of antimycobacterial agents: bioguided fractionation and in vitro activity of alkaloids isolated from *Holarrhena floribunda* used in traditional treatment of buruli ulcer. BioMed Res Int. doi:10.1155/2015/835767

Yotis W, Stanke R (1966) Bacteriostatic action of progesterone on Staphylococci and other microorganisms. J Bacteriol 92:1285–1289

Yu H-H, Kim K-J, Cha J-D, Kim H-K, Lee Y-E, Choi N-Y, You Y-O (2005) Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. J Med Food 8:454–461. doi:10.1089/jmf.2005.8.454

Zerbo P, Millogo-Rasolodimby J, Nacoulma-Ouedraogo OG, Van Damme P (2011) Plantes médicinales et pratiques médicales au Burkina Faso : cas des Sanan. Bois For Trop 1:41–53