PHYTOTHERAPY RESEARCH
Phytother. Res. (2017)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5887

In vitro Study of Five Herbs Used Against Microbial Infections in Burundi

Jérémie Ngezahayo,[1,2]* Sofia Oliveira Ribeiro,[1] Véronique Fontaine,[3] Léonard Hari,[2] Caroline Stévigny[1] and Pierre Duez[1,4]

[1]Laboratoire de Pharmacognosie, Bromatologie et Nutrition Humaine, Faculté de Pharmacie, Université Libre de Bruxelles, CP 205/09, Boulevard du Triomphe, 1050 Bruxelles, Belgium
[2]Centre de Recherche Universitaire en Pharmacopée et Médecine Traditionnelle (CRUPHAMET), Faculté des Sciences, Université du Burundi, BP 2700 Bujumbura, Burundi
[3]Unité de Microbiologie Pharmaceutique et Hygiène, Faculté de Pharmacie, Université Libre de Bruxelles, CP 205/02, Boulevard du Triomphe, 1050 Bruxelles, Belgium
[4]Service de Chimie Thérapeutique et de Pharmacognosie, Université de Mons (UMONS), Bât. 6, Chemin du Champ de Mars 25, 7000 Mons, Belgium

The emergence of antimicrobial resistant infectious diseases remains a major threat to worldwide public health, in developed and in developing countries. Therefore, new antimicrobial agents acting by new mechanisms of action are urgently needed. As plants used in traditional medicine may help to overcome these problems, *Justicia subsessilis*, *Platostoma rotundifolium*, *Pavetta ternifolia*, *Stomatanthes africanus*, and *Virectaria major* (plants highly cited to be used against microbial infections in traditional Burundian medicine) were studied to assess their traditional use efficacy. We conducted a preliminary phytochemical screening of the extracts, as well as their direct and indirect (effect on antibiotic resistance) antibacterial activity on four bacterial strains (*Staphylococcus* sp. and *Escherichia coli*) by broth microdilution methods. All five medicinal plants investigated in this work were found to have direct antibacterial activity against all tested bacterial strains (minimum inhibitory concentration = 62.5–1000 μg/mL) that may support the use of these species in traditional Burundian medicine. Extracts (with no direct antibacterial activity), tested at 200 μg/mL, decreased the MIC values of β-lactams and aminoglycoside antibiotics by a factor of 2 to 64-fold. These interactions between plant extracts and antibiotics could open an avenue of research against antibiotic resistance. Copyright © 2017 John Wiley & Sons, Ltd.

Keywords: Antibiotic resistance; herbal extracts; antibacterial activity; MRSA; FIC index; Burundi.

INTRODUCTION

Infectious diseases currently remain a threat to public health worldwide despite the efforts made in their prevention and treatment. This is due in large part to the appearance of a high percentage of antimicrobial resistant strains among common infections (urinary tract infections, pneumonia, bloodstream infections, etc.) in all regions of the world (WHO, 2015). For example, health centers and hospitals nowadays record high rates of nosocomial infections caused by resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug-resistant Gram-negative bacteria. Another example is the multidrug resistant tuberculosis; 480 000 new cases were identified in 2013, and cases of extensively drug-resistant tuberculosis were acknowledged in 100 countries (WHO, 2015). To fight against such threat, the world is in urgent need of new antimicrobial agents that could have novel mechanisms of action. Moreover, the situation is difficult in many countries where conventional medicine and current advances in infectious disease control are not accessible to most of the population. This is particularly the case in Africa (and other developing countries) where traditional medicine (including use of medicinal plants) remains the primary sources of health care, with one traditional healer for 500 people, against one doctor for 40 000 people (WHO, 2013). In Burundi, numerous plant species are used against infectious diseases. An ethnobotanical survey that we recently conducted in Bujumbura permitted to identify 155 medicinal plants (grouped into 51 families) used against microbial infections in traditional Burundian medicine (Ngezahayo *et al*., 2015). From this survey, five plant species have particularly caught our attention not only by the fact that they were highly cited by traditional healers but also by their quasi-absence in the scientific literature, especially regarding their phytochemistry and biological activities. These are *Justicia subsessilis* (Acanthaceae), *Platostoma rotundifolium* (Lamiaceae), *Pavetta ternifolia* (Rubiaceae), *Stomatanthes africanus* (Asteraceae), and *Virectaria major* (Rubiaceae) whose medical–traditional uses are presented in Table 1.

As part of the valorization of Burundian medicinal plants, and to verify whether the five species (the most cited for their use against microbial infections) are really active *in vitro*, we investigated the phytochemical composition of the plant extracts, as well as their direct antibacterial activity. The influence of the extracts on MRSA resistance to common antibiotics was also evaluated.

* Correspondence to: Jérémie Ngezahayo, Laboratoire de Pharmacognosie, Bromatologie et Nutrition humaine, Faculté de Pharmacie, Université Libre de Bruxelles (ULB), Campus de la Plaine, CP205/9, Boulevard du Triomphe, B-1050 Brussels, Belgium.
E-mail: jngezaha@ulb.ac.be

Contract/grant sponsor: Government of Burundi.

Received 11 March 2017
Revised 16 July 2017
Accepted 18 July 2017

Table 1. Collection areas of the plants and their role in traditional Burundian medicine

Plant names (vernacular names, family, voucher)	Date and harvest area (altitude, position)	Diseases treated in Burundian traditional medicine (parts used)
Justicia subsessilis Oliv. (JS; Umubazibazi, Acanthaceae, BR0000013315863)	9 July 2012; Rutegama in Muramvya Province (1732 m; S 03.316090°, E 029.71669°)	Skin mycosis, varicella, and dysentery (leaves or aerial parts)
Platostoma rotundifolium (Briq.) A. J. Paton (PR; Umusekerasuka, Lamiaceae) BR0000013315900	19 July 2012; Nyabiraba in Bujumbura Rural Province (1730 m, S 03.45325°, E 029.47607°)	Ringworm, purulent rashes, varicella, foot mycosis, diarrhea, fever, cough, and otitis (aerial parts)
Pavetta ternifolia (Oliv.) Hiern (PT; Umunyamabuye, Rubiaceae, 0000013315870)	9 July 2012; Rutegama in Province Muramvya (1743 m; S 03.3625°, E 029.71642°)	Diarrhea and varicella (leaves)
Stomatanthes africanus (Oliv. & Hiern) R. M. King & H. Rob. (SA; Umweyo, Asteraceae, BR0000013284756)	14 July 2013; Kabezi in Bujumbura Rural Province (1051 m; S 03.50182°, E 029.36446°)	Ringworm, purulent rashes, and diarrhea (leaves or aerial parts)
Virectaria major (K. Schum.) Verdc. (VM; Umukizikizi, Rubiaceae, BR0000013315856)	22 July 2012; Kiganda in Muramvya Province (1631 m; S 03.30086°, E 029.72304°)	Ringworm, purulent rashes, cough, fever, diarrhea, measles, yaws, leprosy, and gonorrhea (leaves or aerial parts)

MATERIAL AND METHODS

Collection and identification of plant samples. All plant species (*J. subsessilis*, *P. rotundifolium*, *P. ternifolia*, *S. africanus*, and *V. major*) studied in this work were collected in July 2012 and 2013 in different areas of the Bujumbura Rural and Muramvya Provinces (Burundi; Table 1). Their botanical identification was confirmed by specialists of the herbaria of 'Université du Burundi' and 'Université Libre de Bruxelles' (BLRU), and voucher specimen were deposited in the National Herbarium of Meise (Belgium) under the numbers presented in Table 1.

Chemicals and bacterial strains. Solvents were analytical grade and obtained from VWR International (Leuven, Belgium). All other chemicals used during the phytochemical screening were also analytical grade and purchased from Sigma Aldrich (St Louis, USA). Mueller Hinton broth and agar were from Sigma-Aldrich, whereas physiological solution (NaCl 0.85%, 2 mL) was purchased from BioMerieux (France). Antibiotics (Sigma-Aldrich) such as penicillin G or V, streptomycin, oxacillin, ampicillin, and tetracycline were used as positive controls for microdilution assays, while antibiotic discs (Biolab Inc.) of methicillin (5 μg), cefotaxim (30 μg), oxacillin (1 μg), and penicillin G (5 U) were used for antibiograms. Three Gram-positive (*Staphylococcus* sp.) and one Gram-negative (*Escherichia coli* ATCC 25922) bacterial strains were used in this work. Two *Staphylococcus* strains (C 98506 and C 100459) were MRSA, whereas the third one was susceptible *S. aureus* (MSSA ATCC 6538). Both MRSA strains were clinical isolates from the 'Centre Hospitalier Universitaire de Charleroi' (Belgium), while the ATCC strains were obtained from the American Type Culture Collection.

Plant extract preparation and phytochemical screening. Plant materials (leaves or aerial parts according to traditional use; see Table 2) were dried at room

Table 2. Extraction yields

Plant name[a]	Part of the plant used	Yield (%, m/m) and color of the extract				
		HEX[b]	DCM	ETA	MET	AQ
JS	Aerial parts	1.4% (yellow)	2.4% (black)	0.8% (greenish gray)	5.5% (green)	16.1% (brown)
PR	Aerial parts	1.0% (greenish brown)	3.0% (green)	1.1% (greenish gray)	3.4% (greenish brown)	7.4% (dark red)
PT	Leaves	2.7% (greenish black)	2.3% (greenish white)	0.8% (greenish black)	24.2% (dun)	15.3% (light brown)
SA	Aerial parts	3.6% (greenish yellow)	3.1% (blackish green)	0.4% (blackish green)	6.0% (yellowish brown)	7.8% (brown)
VM	Aerial parts	0.7% (greenish black)	3.5% (white and black mixture)	1.1% (grayish white)	24.4% (greenish)	6.3% (brown)

[a]JS, *Justicia subsessilis*; PT, *Pavetta ternifolia*; PR, *Platostoma rotundifolium*; SA, *Stomatanthes africanus*; VM, *Virectaria major*.
[b]HEX, hexane extract; DCM, dichloromethane extract; ETA, ethyl acetate extract; MET, methanol extract; AQ, aqueous extract.

temperature away from sunlight and then finely ground. Each sample (100 g) was successively percolated with *n*-hexane, dichloromethane, ethyl acetate, methanol, and water; all extracts were evaporated at 40 °C under vacuum. A preliminary phytochemical screening was performed, using standard procedures (Wagner and Bladt, 1996), for the following phytoconstituents: alkaloids (Dragendorff's test), flavonoids (Neu reagent), terpenoids (Liebermann-Burchard reagent), saponins (foam formation), tannins (10% $FeCl_3$ aqueous solution), and anthraquinones (Borntrager's test).

Direct antibacterial assays. Direct (extracts only) and indirect (extracts combined with antibiotics) antibacterial activities were studied by broth microdilution methods as reviewed by Jorgensen and Turnidge (2007). Briefly, in direct microdilution methods, 20 mg of each extract was dissolved in 250 μL of dimethyl sulfoxide (DMSO) and diluted to 5 mL with Mueller-Hinton broth to achieve a final concentration of 5% DMSO. This solution was transferred in 96-well plates (200 μL/well) and diluted with Mueller-Hinton broth. Twenty-four hour culture bacterial strains were stirred with a physiological solution (0.85% NaCl), diluted to McFarland 0.5 turbidity (about 10^8 cells/mL), and inoculated in the 96-well plates. These cultures were then incubated at 37 °C for 24 h in the ambient atmosphere. The minimum inhibitory concentration (MIC) was the lowest concentration of extract that completely inhibited the growth of germs in the microdilution wells, as detected by the unaided eye; the minimal bactericidal concentration (MBC) was determined by subculturing the negative wells on a Mueller-Hinton agar plate and was the lowest concentration that yielded negative subcultures (Okusa *et al*., 2007). Suitable controls were made with media prepared without plant extract but with DMSO (NCCLS, 2003).

Indirect antibacterial assays. Eventual effects on antibiotic resistance were studied by dissolving in DMSO and incorporating in Muller-Hinton broth (200 μg/mL) the extracts that have no direct antibacterial activity. Minimum inhibitory concentrations were determined as described in "Direct antibacterial assays." Positive results were interpreted as a decrease in the MIC of the antibiotics for MRSA in the presence of the plant extracts. Disk diffusion methods were also used to test the sensitivity of bacterial strains to antibiotics (see Fig. S1). In parallel, control tests were also carried out (in the same conditions) with the media prepared without plant extract but with DMSO.

Determination of the interaction between plant extracts and antibiotics. The type of interaction between plant extracts and antibiotics was evaluated by using the Fractional Inhibitory Concentration (FIC) and FIC Index (FICI) values (Mackay *et al*., 2000; Van Vuuren and Viljoen, 2011), which were determined by broth microdilution methods. To study these interactions, only extracts with direct antibacterial activities (MIC ≤ 1000 μg/mL) were combined (in 1:1 ratio) with antibiotics (oxacillin, streptomycin, and tetracycline) and two MRSA strains (C98506 and C100459) were used. Four interaction types were considered in this work: synergy or potentiation, additive or summative effect (additivity), indifference or zero interaction, and antagonism. All these interaction types were evaluated by the following equations:

$$\text{FIC of the antibiotic} = \frac{\text{MIC of the anibiotic in combination with the plant extract}}{\text{MIC of the antibiotic independently}}$$

$$\text{FIC of the plant extract} = \frac{\text{MIC of the plant extract in combination with the antibiotic}}{\text{MIC of the plant extract independently}}$$

$$\text{FICI} = \text{FIC of the antibiotic} + \text{FIC of the plant extract}$$

According to the approach of Mackay *et al*. (2000), the FICI values were classified as follows: synergy (FICI ≤ 0.5), additivity (0.5 < FICI ≤ 1), indifference (1 < FICI ≤ 2), and antagonism (FICI > 2).

RESULTS AND DISCUSSION

Selected plant species and their medical–traditional uses in Africa

Our ethnobotanical survey of medicinal plants used against microbial infections in traditional Burundian medicine (Ngezahayo *et al*., 2015) permitted to select five plants highly cited by traditional healers: *P. rotundifolium* (75% citation), *V. major* (72%), *S. africanus* (59%), *J. subsessilis* (50%), and *P. ternifolia* (5%). Although most of these species were used in combinations with others (multiherbal recipe), this work was first interested in studying the antimicrobial activity of plants used alone (monoherbal recipes) or that were cited as a major ingredient in multiherbal recipes, and/or as an ingredient to be used alone whenever the other plants of the recipe cannot be obtained. In addition to their antimicrobial uses in Burundi (Table 1), these plants are also reported in other African countries for the treatment of cough and diarrhea (*J. subsessilis*; Neuwinger, 2000); fungal and bacterial infections (*P. rotundifolium*; Kamatenesi-Mugisha *et al*., 2008); cough, dysentery, and skin diseases (*P. ternifolia*; Neuwinger, 2000); oral treatments (mouthwash), diarrhea, dysentery, and nasopharyngeal infections (*S. africanus*; Grossi and Katinas, 2013); and pneumonia, eye diseases, and wounds (*V. major*; Dessein *et al*., 2001; Neuwinger, 2000).

Extraction yields and major phytoconstituents

Extraction yields were higher in the most polar extracts (aqueous and methanolic extracts) and lower for other extracts (*n*-hexane, dichloromethane, and ethyl acetate extracts; Table 2). Among the identified major phytoconstituents, the presence of flavonoids and tannins is noteworthy in at least one extract from each plant, as well as the absence of saponins in *S. africanus* (Table 3). It should be reminded that the test reagents lack specificity and that these data need confirming by phytochemical studies. Nevertheless, this preliminary phytochemical screening is in line with the scarce published data on *Justicia* (Corrêa and Alcântara, 2012), *Platostoma* (Aladedunye *et al*., 2008), *Pavetta* (Baldé, 1991a, 1991b, 1991c, 1995a, 1995b, Baldé *et al*., 2015; Idowu *et al*., 2010; Bello *et al*., 2011)*; Stomatanthes* (Bohlmann *et al*., 1981; Bohlmann and Zdero, 1982; Aqil, 1995), and *Virectaria* (Paris and Paris, 1970).

Direct antibacterial activity of plant extracts

All 25 extracts (five extracts by plant) were tested for their direct antibacterial activity. Table 4 indicates that only nine extracts (at least one extract by species) were active, and all other fractions (not indicated in Table 4) were not active. Furthermore, the dichloromethane and ethyl acetate extracts from *P. rotundifolium* exhibited interesting antibacterial activity on all tested strains, especially on the sensitive *S. aureus* ATCC6538 with MIC values down to 62.5 μg/mL. For this species, the MICs of all active extracts coincided with their MBCs, suggesting a bactericidal effect. This species is very promising for its antibacterial effect, classified as 'very interesting' [MICs < 100 μg/mL, according to Rios and Recio (2005)]. Our recent study indicates that this antibacterial activity could be due to acid pentacyclic triterpenoids (Ngezahayo *et al*., 2016).

Some extracts from the other species were classified as 'active' according to the literature (Rios and Recio, 2005) with MICs ranging 250 to 1000 μg/mL. The *J. subsessilis* dichloromethane, methanolic, and aqueous extracts showed MICs comprised between 250 and 500 μg/mL and MBC values generally higher or equal to 1000 μg/mL on all tested strains, suggesting that they have a bacteriostatic effect. This antibacterial activity may be explained by the presence of flavonoids; indeed, it was shown that flavonoids isolated from the genus *Justicia* possess antimicrobial activity (Corrêa and Alcântara, 2012). The *P. ternifolia* dichloromethane extract also exhibited a bacteriostatic effect, especially on MSSA ATCC 6538 and MRSA C100459 strains (MIC = 250 μg/mL and MBC > 1000 μg/mL); this could also be attributed to flavonoids, a major class of secondary metabolites encountered in the genus *Pavetta* (Bello *et al*., 2011) for which an antibacterial activity has already been proven (Cushnie and Lamb, 2005; Pistelli and Giorgi, 2012). The *S. africanus* dichloromethane and ethyl acetate extracts were active with MICs between 500 and 1000 μg/mL and MBC > 1000 μg/mL, suggesting also bacteriostatic effects. These could be

Table 3. Phytochemical screening of plant extracts

Plant	Type of extract[a]	Major phytoconstituents detected[b]					
		Alkaloids	Flavonoids	Terpenoids	Tannins	Saponins[c]	Anthraquinones
J. subsessilis aerial parts	HEX	−	−	+	−	−	−
	DCM	+	±	+	−	−	+
	ETA	+	±	+	−	−	+
	MET	+	+	±	+	−	+
	AQ	−	±	−	+	+ (2.7 cm)	−
Platostoma rotundifolium	HEX	−	−	+	−	−	−
aerial parts	DCM	±	+	+	−	−	+
	ETA	±	+	+	−	−	+
	MET	−	+	+	−	−	+
	AQ	−	−	−	+	+ (3.0 cm)	−
Pavetta ternifolia leaves	HEX	−	−	+	−	−	−
	DCM	−	+	±	±	−	−
	ETA	−	+	−	+	−	−
	MET	−	+	−	+	−	−
	AQ	−	−	−	+	+ (4.0 cm)	−
S. africanus aerial parts	HEX	−	−	+	−	−	−
	DCM	−	−	+	−	−	±
	ETA	−	−	+	−	−	+
	MET	−	−	±	+	−	+
	AQ	−	−	−	−	−	−
V. major aerial parts	HEX	−	+	+	−	−	−
	DCM	±	+	+	−	−	+
	ETA	±	−	−	−	−	+
	MET	+	−	−	−	−	+
	AQ	±	−	−	+	+ (1.5 cm)	−

[a]HEX, hexane extract; DCM, dichloromethane extract; ETA, ethyl acetate extract; MET, methanol extract; AQ, aqueous extract.

[b]+, presence; −, absence; ±, presence in traces.

[c]The number in brackets indicates the height of the formed foam.

Table 4. Direct antibacterial activity of plant extracts

		Minimum inhibitory and bactericidal concentrations (MIC/MBC, μg/mL)							
		Tested bacterial strains							
		MSSA ATCC 6538		*E. coli* ATCC 25922		MRSA C98506		MRSA C100459	
Plants and antibiotics	Type of extracts	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
J. subsessilis aerial parts	DCM	250	1000	500	1000	500	1000	500	1000
	MET	250	1000	500	1000	250	500	250	1000
	AQ	500	1000	500	1000	500	1000	500	1000
Platostoma rotundifolium	DCM	62.5	62.5	1000	1000	125	125	125	125
aerial parts	ETA	62.5	62.5	250	250	125	125	125	125
Pavetta ternifolia leaves	DCM	250	1000	1000	1000	250	1000	1000	1000
S. africanus aerial parts	DCM	500	1000	1000	1000	500	1000	500	1000
	ETA	500	1000	1000	1000	1000	1000	1000	1000
V. major aerial parts	HEX	1000	1000	1000	1000	500	500	500	500
Tetracycline[a]		1	nd	2	nd	1	nd	0.5	nd
Streptomycin[a]		4	nd	4	nd	4	nd	4	nd
Penicillin G[a]		≤0.125	nd	≤0.125	nd	16	nd	8	nd

MSSA, susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; HEX, hexane extract; DCM, dichloromethane extract; ETA, ethyl acetate extract; MET, methanol extract; AQ, aqueous extract; nd, not determined.
[a]Positive control.

Table 5. Screening of plant extracts for their effect on antibiotic resistance

Antibiotics alone and their combination with plant extracts (200 μg/mL)	Minimum inhibitory concentrations (MICs, μg/mL)	
	MRSA C 98506	MRSA C 100459
Penicillin G alone	16	8
Penicillin G + *J. subsessilis* extracts	8–16	2–4
Penicillin G + *P. rotundifolium* extracts	1–2	0.25–0.50
Penicillin G + *P. ternifolia* extracts	8–16	4–8
Penicillin G + *S. africanus* extracts	8–16	4–8
Penicillin G + *V. major* extracts	16	8
Streptomycin alone	4	4
Streptomycin + *J. subsessilis* extracts	2	2–4
Streptomycin + *P. rotundifolium* extracts	0.5–1	0.5–1
Streptomycin + *P. ternifolia* extracts	1–4	2
Streptomycin + *S. africanus* extracts	1	2
Streptomycin + *V. major* extracts	1–2	2

These plant extracts have no direct antibacterial effects (MICs > 1000 μg/mL), and their effect on antibiotic resistance is detailed in Table S1.

Table 6. Effect of *J. subsessilis* and *P. rotundifolium* extracts on resistance of MRSA to β-lactams

Beta-lactams antibiotics alone and their combination with plant extracts (200 μg/mL)	Minimum inhibitory concentrations (MICs, μg/mL)	
	MRSA C 98506	MRSA C 100459
Oxacillin alone	32	8
Oxacillin + *J. subsessilis* extracts	1–16	0.5–1
Oxacillin + *P. rotundifolium* extracts	0.5–1	2
Penicillin V alone	16	8
Penicillin V + *J. subsessilis* extracts	8–16	2
Penicillin V + *P. rotundifolium* extracts	4	1
Ampicillin alone	16	8
Ampicillin + *J. subsessilis* extracts	2–16	2
Ampicillin + *P. rotundifolium* extracts	0.25–8	1–2

These extracts (hexane/ethyl acetate extracts from *J. subsessilis* and hexane/methanol extracts from *P. rotundifolium*) have no direct antibacterial effects (MICs > 1000 μg/mL). Minimum inhibitory concentrations for each extract are detailed in Table S2.

explained by the presence of terpenoids in these extracts; indeed, *Stomatanthes* terpenoids (Bohlmann and Zdero, 1982; Bohlmann *et al*., 1981) are effectively known as antibacterials (Saleem, 2015; Saleem *et al*. 2010). The *V. major* hexane extract showed a bactericidal effect (MIC = MBC = 500–1000 μg/mL), which may be due to flavonoids present in the extract. The genus *Virectaria* is known for some flavonoids (Paris and Paris, 1970) whose antibacterial effect is well established (Cushnie and Lamb, 2005; Pistelli and Giorgi, 2012).

Effect of plant extracts on antibiotic resistance

Sixteen plant extracts (which were inactive in direct antibacterial tests) were evaluated for their effect on antibiotic resistance (Table 5 and details in Table S1). The *n*-hexane and methanol extracts (from *P. rotundifolium*) showed remarkable results, decreasing the MICs of all antibiotics tested on MRSA strains (Tables 5 and 6 and details in Tables S1 and S2), especially β-lactams (penicillin G, from 8 or 16 μg/mL to 0.5 or 2 μg/mL) and

Table 7. Interactions between active plant extracts and antibiotics

Bacterial strains	Combinations	FICI[♣]	Interaction type
MRSA C98506	OXA + JS/DCM	0.19	Synergy
	OXA + JS/MET	0.19	Synergy
	OXA + PR/DCM	0.14	Synergy
	OXA + PT/DCM	0.31	Synergy
	OXA + SA/DCM	0.12	Synergy
	OXA + SA/ETA	0.18	Synergy
	OXA + VM/HEX	0.18	Synergy
	STP + JS/DCM	2.50	Antagonism
	STP + JS/MET	1.25	Indifference
	STP + PR/DCM	0.31	Synergy
	STP + PR/ETA	0.62	Additivity
	STP + PT/DCM	0.37	Synergy
	STP + SA/DCM	0.56	Additivity
	STP + SA/ETA	2.50	Antagonism
	STP + VM/HEX	2.50	Antagonism
	TET + JS/DCM	0.53	Additivity
	TET + JS/MET	0.53	Additivity
	TET + JS/AQ	0.53	Additivity
	TET + PR/DCM	0.15	Synergy
	TET + PT/DCM	0.56	Additivity
	TET + SA/DCM	0.51	Additivity
	TET + SA/ETA	0.53	Additivity
	TET + VM/HEX	0.26	Synergy
MRSA C100459	OXA + JS/DCM	1.50	Indifference
	OXA + JS/MET	1.00	Additivity
	OXA + PR/DCM	1.25	Indifference
	OXA + PT/DCM	0.62	Additivity
	OXA + SA/DCM	2.50	Antagonism
	OXA + SA/ETA	1.50	Indifference
	OXA + VM/HEX	2.50	Antagonism
	STP + JS/DCM	0.62	Additivity
	STP + JS/MET	0.62	Additivity
	STP + PR/DCM	0.62	Additivity
	STP + PR/ETA	0.62	Additivity
	STP + PT/DCM	0.56	Additivity
	STP + SA/DCM	0.56	Additivity
	STP + SA/ETA	0.62	Additivity
	STP + VM/HEX	0.56	Additivity
	TET + JS/DCM	0.62	Additivity
	TET + JS/MET	0.62	Additivity
	TET + PR/DCM	0.56	Additivity
	TET + PT/DCM	1.00	Additivity
	TET + SA/DCM	1.00	Additivity
	TET + SA/ETA	1.00	Additivity
	TET + VM/HEX	0.50	Synergy

FICI, Fractional Inhibitory Concentration Index; OXA, oxacillin; STP, streptomycin; TET, tetracycline; JS/DCM, JS/MET, and JS/AQ, dichloromethane, methanol, and aqueous extracts from *J. subsessilis*; PR/DCM and PR/ETA, dichloromethane and ethyl acetate extracts from *P. rotundifolium*; PT/DCM, dichloromethane extract from *P. ternifolia*; SA/DCM and SA/ETA, dichloromethane and ethyl acetate extracts from *S. africanus*; VM/HEX, hexane extract from *V. major*.

[♣]Details on calculation of FICI values are presented in Table S3.

aminoglycoside (streptomycin, from 4 to 0.5–1 μg/mL) antibiotics. The *J. subsessilis* extracts also decreased the MIC values of penicillin G (two-fold to eight-fold) and streptomycin (only two-fold). The extracts of *P. ternifolia*, *S. africanus*, or *V. major* weakly modulated the activity of streptomycin, whose MICs decreased by a factor of two-fold to four-fold. These extracts were also tested with other β-lactams (Table 6 and details in Table S2) on the same MRSA strains. The *n*-hexane and methanol extracts from *P. rotundifolium* decreased the MICs of oxacillin (from 8 or 32 to 0.5 or 2 μg/mL), penicillin V (from 8 or 16 to 1 or 4 μg/mL), and ampicillin (from 8 or 16 to 0.25 or 1 μg/mL) for both tested strains. Similarly, the *n*-hexane and ethyl acetate extracts from *J. subsessilis* decreased the MICs of oxacillin (4 to 64-fold), penicillin V (2 to 8-fold), and ampicillin (2 to 8-fold).

Considering that these *P. rotundifolium* and *J. subsessilis* extracts are inactive in direct antimicrobial tests, the observed resistance-reversion effects, which considerably enhance the activity of β-lactams and aminoglycoside antibiotics on MRSA, are particularly interesting and worthy of further investigations.

Interactions between plant extracts and antibiotics

The nine extracts having demonstrated bactericidal or bacteriostatic effect were combined with β-lactams (oxacillin), aminoglycoside (streptomycin), and tetracycline antibiotics to evaluate their eventual interactions (synergy, additivity, indifference, or antagonism) against two MRSA strains C98506 and C100459. Of all 45 tested combinations, 5 antagonism cases and 4 indifference cases were observed. The other combinations were either synergistic (12) or additive (24) cases (Table 7 and details in Table S3). The most favorable synergistic interactions were observed with oxacillin in combination with the dichloromethane (FICI = 0.12) or ethyl acetate (FICI = 0.18) extracts from *S. africanus* on the one hand and with the dichloromethane (FICI = 0.14) extract from *P. rotundifolium* on the other hand, especially against the strain MRSA C98506. As these interesting herbs have practically not been investigated, it is difficult to make comparisons with data from the literature; it is noteworthy that both extracts contain terpenoids (Table 3) and members of this chemical family are known both for their modulating properties of bacterial multiresistances (Gibbons *et al*., 2003; Oluwatuyi *et al*., 2004) and for synergistic effects with β-lactams (including methicillin) by interference with penicillin binding proteins 2a (PBP2a; Nicholson *et al*., 1999).

The *J. subsessilis* dichloromethane and methanol extracts also presented interesting synergistic effects with oxacillin (FICI = 0.19), which might be attributed to their content in flavonoids. Synergistic effects of flavonoids with β-lactams (including oxacillin) are effectively known in literature (Hatano *et al*., 2005).

CONCLUSIONS

All five medicinal plants investigated in this work were found to have direct antibacterial activity against all tested bacterial strains, which may support the use of these species in the treatment of microbial infections in traditional Burundian medicine. Bactericidal or bacteriostatic extracts also showed synergistic interactions with antibiotics such as oxacillin (β-lactam), streptomycin (aminoglycoside), and tetracycline, thus increasing the therapeutic importance of each species in the antibacterial combat. Interestingly, some extracts without direct antibacterial effect (MICs > 1000 μg/mL) permitted to reduce the MIC of β-lactams (penicillin G and V, oxacillin, and ampicillin) and aminoglycoside (streptomycin) antibiotics by a factor of 2 to 64-fold.

Of the five medicinal plants investigated, *P. rotundifolium* was the most active and the most pharmacologically interesting. Indeed, the dichloromethane and ethyl acetate extracts from this species exhibited a promising direct antibacterial activity (MIC = 65.5–250 μg/mL), corroborating our ethnobotanical survey data that, with 75% of citations, placed this species in first position of the medicinal plants used by Burundian traditional healers for the treatment of microbial diseases (Ngezahayo *et al*., 2015). The *n*-hexane and methanol extracts from this species were also quite active in reducing the MICs of four β-lactams and one aminoglycoside on MRSA, suggesting the suppression of antibiotic resistance. Finally, the fact that these extracts enhance the activity of certain antibiotics against MRSA could open an avenue of research against antibiotic resistance.

Acknowledgements

Mrs Marie Faes and M. Olivier Vaillant are acknowledged for their role in maintaining excellent laboratory working conditions. This work was partly funded by the Government of Burundi and the Xenophilia Fund of the 'Université Libre de Bruxelles' (ULB). It was also partly supported by the 'Académie de Recherche et d'Enseignement supérieur' (ARES) as well as 'Le Fonds De Meurs-François' (ULB).

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Aladedunye FA, Okorie DA, Ighodaro OM. 2008. Anti-inflammatory and antioxidant activities and constituents of *Platostoma africanum* P. Beauv. *Nat Prod Res* **22**(12): 1067–1073.

Aqil M. 1995. Flavonoidic glycosides from *Eupatorium africanum*. *Ultra Sci Phys Sci* **7**(1): 1–6.

Baldé AM, Pieters LA, Gergely A, Kolodziej H, Claeys M, Vlietinck AJ. 1991a. A-type proanthocyanidins from stembark of *Pavetta owariensis*. *Phytochemistry* **30**(1): 337–342.

Baldé AM, Claeys M, Pieters L, Wray V, Vlietinck A. 1991b. Ferulic acid esters from stembark of *Pavetta owariensis*. *Phytochemistry* **30**(3): 1024–1026.

Baldé AM, Pieters LA, Wray V, *et al*. 1991c. Dimeric and trimeric proanthocyanidins possessing a doubly linked structure from *Pavetta owariensis*. *Phytochemistry* **30**(12): 4129–4135.

Baldé AM, Pieters LA, Claeys M, *et al*. 2015. Quinic acid esters from *Pavetta owariensis* var. owariensis (Rubiaceae). *J Plant Sci* **3**: 20–23.

Baldé AM, de Bruyne T, Pieters L, *et al*. 1995a. Oligomeric proanthocyanidins possessing a doubly linked structure from *Pavetta owariensis*. *Phytochemistry* **38**(3): 719–723.

Baldé AM, de Bruyne T, Pieters L, *et al*. 1995b. Tetrameric proanthocyanidins containing a double interflavanoid (A-type) linkage from *Pavetta owariensis*. *Phytochemistry* **40**(3): 933–938.

Bello IA, Ndukwe GI, Audu OT, Habila JD. 2011. A bioactive flavonoid from *Pavetta crassipes* K. Schum. *Org Med Chem Lett* **1**(14): 1–5.

Bohlmann F, Zdero C. 1982. A dehydrofarnesol derivative from *Stomanthes africanus*. *Phytochemistry* **21**(5): 1155–1156.

Bohlmann F, Zdero C, Fiedler L, Robinsons H, Kings RM. 1981. A labdane derivative from Chromolaena collina and a *p*-hydroxyacetophenone derivative from *Stomatanthes corumbensis*. *Phytochemistry* **20**(5): 1141–1143.

Corrêa GM, Alcântara AFC. 2012. Chemical constituents and biological activities of species of Justicia—a review. *Rev. Bras. Farmacogn Braz J Pharmacogn* **22**(1): 220–238.

Cushnie TPT, Lamb AJ. 2005. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* **26**: 343–356.

Dessein S, Jansen S, Huysmans S, Robbrecht E, Smets E. 2001. A morphological and anatomical survey of Virectaria (African Rubiaceae), with a discussion of its taxonomic position. *Bot J Linn Soc* **137**: 1–29.

Gibbons S, Oluwatuyi M, Veitch NC, Gray AI. 2003. Bacterial resistance modifying agents from Lycopus europaeus. *Phytochemistry* **62**: 83–87.

Grossi MA, Katinas L. 2013. A new circumscription of the genus *Stomatanthes* (Asteraceae, Eupatorieae). *Syst Bot* **38**(3): 830–849.

Hatano T, Kusuda M, Inada K, *et al*. 2005. Effects of tannins and related polyphenols on methicillin-resistant *Staphylococcus aureus*. *Phytochemistry* **66**: 2047–2055.

Idowu TO, Ogundaini AO, Salau AO, Obuotor EM, Bezabih M, Abegaz BM. 2010. Doubly linked, A-type proanthocyanidin trimer and other constituents of Ixora coccinea leaves and their antioxidant and antibacterial properties. *Phytochemistry* **71**: 2092–2098.

Jorgensen JH, Turnidge JD. 2007. Susceptibility test methods: dilution and disk diffusion methods. In Manual of Clinical Microbiology (9th Edn), Murray PR, Baron EJO, Jorgensen JH, Landry ML, Pfaller MA (eds). ASM Press: Washington.

Kamatenesi-Mugisha M, Oryem-Origa H, Odyek O, Makawiti DW. 2008. Medicinal plants used in the treatment of fungal and bacterial infections in and around Queen Elizabeth Biosphere Reserve, western Uganda. *Afr J Ecol* **46**(Suppl. 1): 90–97.

Mackay ML, Milne K, Gould IM. 2000. Comparison of methods for assessing synergic antibiotic interactions. *Int J Antimicrob Agents* **15**: 125–129.

NCCLS. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard, sixth ed. NCCLS document M7-A6, Wayne.

Ngezahayo J, Havyarimana F, Hari L, Stévigny C, Duez P. 2015. Medicinal plants used by Burundian traditional healers for the treatment of microbial diseases. *J Ethnopharmacol* **173**: 338–351.

Ngezahayo J, Pottier L, Ribeiro SO, *et al*. 2016. Plastotoma rotundifolium aerial tissue extract has antibacterial activities. *Ind Crop Prod* **86**: 301–310.

Nicholson K, Evans G, O'Toole PW. 1999. Potentiation of methicillin activity against methicillin resistant *Staphylococcus aureus* by diterpenes. *FEMS Microbiol Lett* **179**: 233–239.

Neuwinger HD. 2000. African Traditional Medicine: A Dictionary of Plant Use and Applications. Medpharm Scientific Publishers: Stuttgart.

Okusa PN, Penge O, Devleeschouwer M, Duez P. 2007. Direct and indirect antimicrobial effects and antioxidant activity of Cordia gilletii De Wil (Boraginaceae). *J Ethnopharmacol* **112**: 476–481.

Oluwatuyi M, Kaatz GW, Gibbons S. 2004. Antibacterial and resistance modifying activity of Rosmarinus officinalis. *Phytochemistry* **65**: 3249–3254.

Paris M, Paris RR. 1970. Polyphenols of western Africa Virectaria multiflora, Rubiaceae. *Plantes Medicinales et Phytotherapie* **4**(3): 225–229.

Pistelli L, Giorgi I. 2012. Antimicrobial properties of flavonoids. In Dietary Phytochemicals and Microbes, Patra AK (ed). Springer: India.

Saleem M. 2015. Natural products as antimicrobial agents—an update. In Novel Antimicrobial Agents and Strategies, Phoenix DA, Harris F, Dennison SR (eds). Wiley: Weinheim.

Saleem M, Nazir M, Ali MS, *et al*. 2010. Antimicrobial natural products: an update on future antibiotic drug candidates. *Nat Prod Rep* **27**: 238–254.

Rios JL, Recio MC. 2005. Medicinal plants and antimicrobial activity. *J Ethnopharmacol* **100**: 80–84.

Van Vuuren S, Viljoen A. 2011. Plant-based antimicrobial studies—methods and approaches to study the interaction between natural products. *Planta Med* **77**: 1168–1182.

Wagner H, Bladt S. 1996. Plant Drug Analysis: A Thin Layer Chromatography Atlas, 2nd edn. Berlin: Springer.

WHO. 2013. Traditional Medicine Strategy 2014–2023. WHO Press: Geneva.

WHO, 2015. Antimicrobial resistance, http://www.who.int/mediacentre/factsheets/fs194/en/, accessed 15th May 2016.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.