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In Vitro Evaluation of the Antibacterial and Antioxidant Activities of Extracts From Five Medicinal Plants Traditionally Used to Treat Infections in Burundi

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Abstract

Infectious diseases periodically emerge or re-emerge, causing epidemics or pandemics that significantly impact global health. While hygiene measures and antibiotics have improved infection control, the rapid spread of antimicrobial resistance remains a major threat. In this context, extracts from five plants used in traditional Burundian medicine (*Mikania natalensis* DC., *Helichrysum congolanum* Schltr. & O. Hoffm., *Justicia nyassana* Lindau, *Urtica massaica* Mildbr., and *Senecio maranguensis* O. Hoffm.) were evaluated for their antibacterial and antioxidant potential. Antibacterial activities were assessed on fifteen bacterial strains using microdilution method and TLC-bioautography, while antioxidant activity was assessed through the DPPH$^{\bullet}$ radical scavenging method. Gram-positive strains, particularly *S.*

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aureus, showed greater sensitivity compared to Gram-negative bacteria. Four plants exhibited active extracts with MICs between 250 and 1000 µg/mL, except for *S. maranguensis* (MICs ≥ 2000 µg/mL). *M. natalensis* was the most active, with dichloromethane and ethyl acetate extracts showing MICs of 250 and 500 µg/mL. Beyond their intrinsic antibacterial activity, this study reveals that otherwise inactive extracts of *M. natalensis* can significantly potentiate β-lactam and aminoglycoside antibiotics against multidrug-resistant *S.aureus*, highlighting a previously underexplored resistance-modulating effect. Methanolic extracts of all five plants displayed modest antioxidant activity, with IC_{50} values, expressed as quercetin equivalents (IC_{50} QE) ranging from < 0.08 to 0.169±0.0065. These findings highlight the potential of Burundian medicinal plants in combating antibiotic resistances, though the use of *S. maranguensis* should be carefully reevaluated, given the well-known occurrence of genotoxic and hepatotoxic pyrrolizidine alkaloids in the genus.

Keywords: Antibacterial activity, antioxidant activity, Methicillin-Resistant *Staphylococcus aureus*, Mikania natalensis, Burundi herbal medicine

Introduction

Infectious diseases, caused by pathogenic microorganisms, spread among individuals and populations, posing serious threats to both public health and the economy (Chen et al., 2019). Periodically, over centuries, infectious diseases emerge or re-emerge, causing epidemics or pandemics that decimate populations worldwide and disrupt social organizations (Raoul & Yazdanpanah, 2022). These threats vary in their severity and likelihood with widely varying consequences for morbidity, mortality as well as society and the economy (Bloom & Cadarette, 2019); this major public health problem has, in recent decades, taken on new proportions and characteristics (Aspect et al., 2021). Indeed, although the overall mortality and morbidity from infectious diseases have declined over the last century, thanks to advances in medicine, improved hygiene and sanitation, access to healthcare, antibiotic discovery and large-scale vaccination programs (Baker et al., 2022; Sorci & Faivre, 2023), the emergence of new pathogens resistant to currently available antimicrobial agents has revived the risk of uncontrolled infections (Aspect et al., 2021), as a global threat (Getahun et al., 2023; Sorci & Faivre, 2023). To combat this phenomenon and thus reduce and/or eradicate mortality from infectious diseases, the world is constantly looking for new antimicrobial agents that could have new mechanisms of action (Murray et al., 2022).

In low- and middle-income countries, notably in Sub-Saharan Africa, the low availability of quality health care, combined with the emergence of resistances, means that the burden of infectious diseases remain high, the death toll linked to emerging and re-emerging infections adding to seasonal and

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endemic infections (Baker et al., 2022); as the cost of medical treatments is often beyond the reach of African people, traditional medicine still has a major position in healthcare (Diatta et al., 2022), with a ratio of 500 people for one traditional healer, compared to 40,000 people for one medical doctor (WHO, 2013). The many properties of medicinal plants used to treat infections, all over the world, generate a growing interest in their use as a possibly safe and renewable alternative to the current antibiotic molecules (Diatta et al., 2022; Rodrigues et al., 2019).

In an ethnobotanical survey conducted in the city of Bujumbura (Burundi) from 2011 to 2013, 155 medicinal plants used in traditional Burundian medicine for the treatment of *"diseases compatible with a microbial infection"*, i.e. probably infectious diseases, were identified (Ngezahayo et al., 2015). In our previous study, the local usages of 5 of the medicinal plants widely cited by Burundian healers (the Urticaceae *Urtica massaica* Mildbr., the Asteraceae *Mikania natalensis* DC., *Senecio maranguensis* O. Hoffm. and *Helichrysum congolanum* Schltr. & O. Hoffm., and the Acanthaceae *Justicia nyassana* Lindau) were exhaustively investigated as well as their chromatographic characterization (Nzoyisubiziki et al., 2024). Given the abundant phenolic compounds we detected in these five plants (Nzoyisubiziki et al., 2024) and the overwhelming evidence of the role of oxidative stress in acute and chronic infections (Ivanov et al., 2017), the present study aims to assess not only the antibacterial but also the antioxidant activities of their extracts.

Material and Methods

Plant material

Three of the five plants (*J. nyassana*, *H. congolanum* and *M. natalensis*) were collected in Ruyigi province, the other two (*U. massaica* and *S. maranguensis*) in Rumonge province. Details of the dates and locations of sample collection are provided in Table 1. Geographical coordinates were measured with a Garmin Oregon 750 GPS.

Table 1: Plant names, locations and dates of harvest

N°	**Plant name (vernacular name, "voucher")**	**Parts used**	**Harvesting location (Position)**	**Harvesting date**
1	*Mikania natalensis* (Nkurimwonga, "NJA003")	Aerial parts (leaves and stems)	Kizigama Hill, Butaganzwa Commune, Ruyigi Province (1569 m; 3°28'05.22'' S, 30°09'16.08'' E)	February 07, 2019
2	*Helichrysum congolanum* (Ngabimwe, "NJA005")	Leaves	Biyorwa Hill, Butaganzwa Commune, Ruyigi Province	February 07, 2019

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			(1508 m; 3°27'29.46'' S, 30°05'30.30'' E)	
3	*Justicia nyassana* (Ikinga, "NJA001")	Leaves	Biyorwa Hill, Butaganzwa Commune, Ruyigi Province (1508 m; 3°27'29.40'' S, 30°05'30.42'' E)	February 07, 2019
4	*Senecio maranguensis* (Imbatura, "NJA004")	Leaves	Zingi-Nyaruyaga Hill, Bugarama Commune, Rumonge Province (1941 m; 3°42'58.38'' S, 29°27'36.78'' E)	February 23, 2019
5	*Urtica massaica* (Igisuru, "NJA002")	Aerial parts (leaves and stems)	Zingi-Nyaruyaga Hill, Bugarama Commune, Rumonge Province (2033 m; 3°42'55.86'' S, 29°27'55.86'' E)	February 23, 2019

Bacterial strains

Fifteen bacterial strains, including *(i)* 8 Gram (+) strains: Methicillin-sensitive *S. aureus* (MSSA) CNR 21248, CNR 21254, LMG 8064, LMG 15975 and LMG 16217; methicillin-resistant *S. aureus* (MRSA) CNR 21249, CNR 21253 and C100459; and *(ii)* seven Gram (-) strains: 4 *Escherichia coli* strains (ATCC 25922, ATCC 35218, ATCC 27553, LMG 15862), one *Klebsiella pneumoniae* strain (LMG 20218), one *Enterobacter aerogenes* strain (LMG 2094) and one *Pseudomonas aeruginosa* strain (LMG 1242) were used in this study. The LMG type strains were supplied by the LMG Bacteria Collection of the University of Ghent and the rest were obtained from the *S. aureus* National Reference Centre of the Erasme Hospital in Brussels (Belgium).

Methods

Obtaining of plant extracts

Plant samples were dried in open air, protected from direct sunlight, then ground in a mortar and sieved through a 1-mm mesh to obtain a fine powder. Successive macerations were performed on 150 g of plant powder for 48 h with five solvents of increasing polarities (1.5 L each): n-heptane (99+%, Acros Organics), dichloromethane (stabilized with 0.2% ethanol, for analysis, VWR), ethyl acetate (for analysis, VWR), methanol (for analysis, Merck), and water. The use of solvents with progressive polarities was intended to maximize the extraction of metabolites with different physicochemical properties, while the 48-h duration ensured adequate diffusion and solubilization of compounds (Bekro et al., 2007; Handa et al., 2008; Ngezahayo et al., 2017). Organic extracts were concentrated using a rotary

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evaporator at 40°C, and aqueous extracts were freeze-dried. This procedure yielded 25 extracts in total (five per plant).

Determination of the antibacterial activity of plant extracts

Preparation of the bacterial inoculum

After incubating the bacteria for 18 to 24 h on a culture medium prepared from a mixture of 72.7% Tryptic Soy Broth (TSB) and 27.3% Agar, the bacterial inoculum was prepared by diluting isolated colonies in physiological water to obtain a bacterial suspension adjusted to an absorbance ranging 0.08 to 0.10 at a wavelength of 600 nm using a Jenway 7200 spectrophotometer. This bacterial suspension corresponds to the McFarland 0.5 standard (~ 10^8 bacteria/mL).

Preparation of plant extracts solutions

For each plant extract, 20 mg were suspended in 250 µL of dimethyl sulphoxide (DMSO) and diluted to 5 mL with Mueller Hinton Broth (MHB) to yield a concentration of 5% DMSO and 4 mg/mL extract.

Antibacterial test by a microdilution method

For antibacterial activity testing, a microdilution method was used as previously described (Ngezahayo et al., 2017; Okusa et al., 2010). Briefly, each extract or antibiotic solution was transferred to a 96-well plate (200 µL/well) followed by logarithmic 2-fold dilutions with Mueller Hinton broth. Afterwards, 100 µL of the 0.5 MacFarland bacterial suspension was inoculated into 88 wells (8 wells serving as negative controls) already containing 100 µL of extract or antibiotic dilutions. The plates were then incubated at 37°C between 18h and 24h. After adding 50 µL of a 0.8 mg/mL solution of 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium (MTT) to each well and incubating for 30 min, the minimum inhibitory concentration (MIC) was detected by unaided eye as the lowest concentration of extract that completely inhibited bacterial growth. The minimum bactericidal concentration (MBC), which is the lowest concentration of the extract that kills bacteria, was determined by sub-culturing the negative wells on a Mueller-Hinton agar plate. All assays (MIC, MBC) were performed in triplicate independent experiments. As identical MIC and MBC values were consistently obtained in all replicates, results are presented as single values. According to (Rios & Recio, 2005), extracts with MICs below 1000 µg/mL were considered as *"positive activity"*, and those with MICs below 100 µg/mL as *"highly promising"*.

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Bio-autography test

Thin layer chromatography (TLC) was performed following the procedure described by (Bekro et al., 2007), with some adjustments. The chromatoplates were aluminum-backed plates precoated with silica gel 60 F254 (Merck KGa, 64271 Darmstadt, Germany). Samples were manually applied at a volume of 10 µL per spot, with an extract concentration of 4 mg/mL (i.e. 40 µg/spot). The TLC plates were developed with n-hexane-ethyl acetate (80 :20, v/v), over 10 cm in a saturated chromatographic N-chamber. After development, the plates were either used for bio-autography or sprayed with anisaldehyde and sulfuric acid, with visualization under visible and 366 nm lights.

For TLC bio-autography, a mixture of MHB/(TSB+Agar) culture media in the proportions (9:1) was prepared, equilibrated at 50°C (Okusa et al., 2010), and seeded with a 0.5 McFarland bacterial suspension in the proportions (9:1). After migration of the TLC and thorough evaporation of the solvents, the bacterial suspension was distributed, rapidly and evenly, on the plate that was then incubated at 37°C for 18-24 h. A sterile solution of MTT (0.8 mg/ml) was then sprayed onto the plate, which was reincubated at 37°C for 2 hours. The areas observed as light spots on a purple or dark background indicate inhibition of bacterial growth by the active substances present on the plate.

Study of the effect of M. natalensis extracts on antibiotic resistance

As *M. natalensis* extracts were interesting for their widespread activities (Table 3), eventual synergistic effects with beta-lactam and aminoglycoside antibiotics were investigated on 3 MRSA strains (CNR 21249, CNR 21253 and C100459). The extracts of this plant (200 µg/mL), which were inactive on these strains (MIC > 1000 µg/mL), were combined with the antibiotics in the proportions (1 :1) and the assay was carried out using the microdilution method under the same conditions as for the MIC determination. The concentration of 200 µg/mL was selected for all extracts as a sub-inhibitory concentration, allowing the assessment of antibiotic potentiation without intrinsic antibacterial effects.

Antioxidant activity

A series of concentrations (12.5 to 300 µg/mL) were prepared for the methanolic extracts, and for the reference compounds, quercetin and ascorbic acid. For each concentration, 100 µL of sample were mixed with 200 µL of a 0.04 % solution of 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) in methanol and incubated in the dark for 15 min. Absorbances were measured at 490 nm using a BioTeck ELx808 96-well microplate reader against a blank

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(100 µL methanol and 200 µL DPPH•). The percentage of DPPH• quenching was calculated according to Equation 1.

$$\textbf{\textit{Percentage of DPPH• quenching}}\ (\%) = \frac{\boldsymbol{A_0 - A_1}}{\boldsymbol{A_0}}\ \boldsymbol{x\ 100} \quad (\mathbf{1})$$

Where: A_0 = Absorbance of the blank; A_1 = Absorbance of the sample.
Linear or semi-log graphs allowed to extrapolate the capacity to reduce the DPPH• radical in IC_{50} values; these were expressed in quercetin equivalents (IC_{50} QE) according to Equation 2.

$$\boldsymbol{IC_{50}\ QE} = \frac{\boldsymbol{IC_{50}\ of\ quercetin\ (mg.mL^{-1})}}{\boldsymbol{IC_{50}\ of\ extract\ (mg.mL^{-1})}} \quad (\mathbf{2})$$

with IC_{50} of quercetin and IC_{50} of extract extrapolated from their respective DPPH• quenching curves. All DPPH assays were performed in triplicate independent experiments, and results are expressed as mean values ± standard deviation (SD).

Results and discussion

Extraction yields

Extraction yields are summarized in

Table 2. As expected for leaves and aerial parts, the most polar solvents (methanol and water) gave the highest yields, ranging from 7.35–25.03% and 10.31–18.57%, respectively, across all studied plants. In contrast, non-polar and moderately polar solvents gave substantially lower yields: 1.20–2.82% for n-heptane, 2.15–5.22% for dichloromethane, and 0.75–5.65% for ethyl acetate. These results follow the expected polarity-dependent extraction pattern reported in previous studies (Ngezahayo et al., 2017).

Table 2 : Extraction yield for each plant and solvent

Plant name	Parts used	Extraction yield (%, w/w)				
		Hept	**Di**	**Ac**	**Me**	**Aq**
Mikania natalensis	Aerial parts (leaves and stems)	2.82	5.22	2.00	7.44	18.57
Helichrysum congolanum	Leaves	1.35	2.70	5.65	13.70	11.12
Justicia nyassana	Leaves	2.00	2.15	0.75	7.35	10.31
Senecio maranguensis	Leaves	1.37	3.13	5.63	25.03	15.34
Urtica massaica	Aerial parts (leaves and stems)	1.20	2.27	1.07	14.20	13.50

Hept : heptane ; **Di** : dichloromethane ; **Ac** : ethyl acetate ; **Me** : methanol ; **Aq** : water

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Determination of antibacterial activities

Four of the 5 plants studied have at least one extract active against at least one of the 15 bacterial strains tested. All the extracts were active against Gram (+) bacteria, notably against *Staphylococcus aureus* strains, but only slightly active or inactive against Gram (-) bacteria (**Error! Reference source not found.**).

Table 3 : Antibacterial activity of extracts from the 5 investigated plants

Plant name, part used	**Extract**	**Minimum inhibitory concentration (MIC) in µg/mL**														
		Gram +								**Gram -**						
		S. aureus CNR 21249 ***(MRSA)***	*S. aureus* CNR 21253 ***(MRSA)***	*S. aureus* C 100459 ***(MRSA)***	*S. aureus* CNR 21248	*S. aureus* CNR 21254	*S. aureus* LMG 16217	*S. aureus* LMG 8064	*S. aureus* LMG 15975	*E. coli* ATCC 25922	*E. coli* ATCC 35218	*E. coli* ATCC 27553	*E. coli* LMG 15862	*E. aerogenes* LMG 2094	*P. aeroginosa LMG 1242*	*K. pneumoniae* LMG 20218
Mikania natalensis, aerial parts	MN/Hept	>2000	500	>2000	1000	500	1000	500	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	MN/Di	500	250	500	250	250	500	250	500	>2000	>2000	1000	>2000	>2000	>2000	>2000
	MN/Ac	500	500	500	500	500	1000	1000	500	>2000	>2000	>2000	>2000	>2000	Nd	>2000
	MN/Me	>2000	>2000	2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	MN/Aq	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
Helichrysum congolanum, Leaves	HC/Hept	1000	2000	2000	1000	1000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	HC/Di	1000	1000	1000	2000	2000	>2000	>2000	2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	HC/Ac	250	500	2000	250	1000	>2000	>2000	>2000	>2000	>2000	2000	>2000	>2000	Nd	>2000
	HC/Me	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	HC/Aq	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
Justicia nyassana, Leaves	JN/Hept	>2000	>2000	>2000	>2000	>2000	>2000	1000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	JN/Di	>2000	>2000	>2000	>2000	>2000	>2000	250	>2000	>2000	>2000	>2000	>2000	Nd	>2000	>2000
	JN/Ac	>2000	>2000	>2000	>2000	>2000	>2000	1000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	JN/Me	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	JN/Aq	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
Senecio maranguensis, Leaves	SM/Hept	2000	>2000	2000	2000	Nd	>2000	2000	2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	SM/Di	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	SM/Ac	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	Nd	Nd	>2000
	SM/Me	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	SM/Aq	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	UM/Hept	>2000	>2000	>2000	>2000	>2000	>2000	1000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000

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Urtica massaica, aerial parts	UM/Di	>2000	>2000	>2000	1000	1000	>2000	1000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	UM/Ac	>2000	>2000	>2000	>2000	>2000	Nd	250	>2000	>2000	>2000	>2000	>2000	Nd	Nd	>2000
	UM/Me	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	Nd	>2000	>2000
	UM/Aq	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
Streptomycin		8	> 64	8	8	8	Nd	4	8	16	16	8	>128	8	4	4
Penicillin G		16	8	16	2	1	32	0.5	16	64	64	64	>128	Nd	>128	Nd

MN : *Mikania natalensis* ; HC : *Helichrysum congolanum* ; UM : *Urtica massaica* ; SM : *Senecio maranguensis* ; JN : *Justicia nyassana* ; Hept : Heptane ; Di : Dichloromethane ; Ac : ethyl acetate ; Me : Methanol ; Aq : Aqueous (successive extractions with increasing polarity solvents); Nd: Not determined due to material limitations.

These results indicate that the most active fractions were the dichloromethane extract of *M. natalensis* (MN/Di), which inhibited 8 *S. aureus* strains with MIC values ranging from 250 to 500 µg/mL; the ethyl acetate extract of *M. natalensis* (MN/Ac), active against 6 strains with a MIC of 500 µg/mL; the ethyl acetate extract of *H. congolanum* (HC/Ac), which exhibited MICs between 250 and 500 µg/mL against 3 strains; and the dichloromethane extract of *J. nyassana* (JN/Di), which showed an MIC of 250 µg/mL against one strain. With an MBC/MIC ratio of less than 4, these extracts demonstrate bactericidal activity against the tested *S. aureus* strains (Wong, 2025)Table 4).

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Table 4: Summary of best-performing extracts and key antibacterial outcomes

Plant species	Extract	Taget strain	MIC (µg/Ml)	MBC (µg/Ml)	MBC/MIC
M. natalensis	MN/Di	CNR 21253	250	500	2
		CNR 21248			2
		CNR 21254			2
		CNR 21249	500		1
		C 100459			1
		LMG 16217			1
		LMG 15975			1
	MN/Ac	CNR 21249	500	1000	2
		CNR 21253			2
		CNR 21248			2
		CNR 21254			2
		C 100459			2
		LMG 15975			2
H. congolanum	HC/Ac	CNR 21248	250	500	2
		CNR 21249			2
		CNR 21253	500	1000	2
J. nyassana	JN/Di	LMG 8064	250	1000	4

Regarding *Mikania natalensis*, none of the extracts were active against tested Gram (-) bacterial strains. The dichloromethane extract (MN/Di) *(i)* was active on all eight *S. aureus* strains, MICs ranging from 250 to 500 µg/mL with MBC 500 µg/mL, i.e. an essentially bactericidal action; and *(ii)* on an *E. coli* strain (*E. coli* ATCC 27553) with a MIC of 1000 µg/mL (MBC > 2000). The ethyl acetate extract (MN/Ac) was also active on *S. aureus* strains (MICs 500 to 1000 µg/mL; MBC, 1000 µg/mL). The other extracts of *M. natalensis* (heptane, methanol, water) were considered inactive. Although the *M. natalensis* species has not been previously investigated for its antimicrobial activities, other species of the *Mikania* genus are reported for antibacterial activities. Indeed, the aqueous and methanolic extracts of *M. micrantha* Kunth were active against *E. coli*, *B. subtilis*, *S. aureus* and *P. vulgaris* (Da Silva et al., 2018); the hexane extract of *M. glomerata* Spreng. significantly inhibited the MRSA strain PI57 (Da Silva et al., 2018; Rufatto et al., 2012). Various extracts of *M. micrantha* Kunth have shown antibacterial activities against multi-drug resistant pathogens, both Gram (-) and Gram (+), including *P. aeruginosa*, *Salmonella typhi*, *S. aureus*, *S. epidermidis*, *B. subtilis*, *E. coli* and *Streptococcus pneumoniae* (Sheam et al., 2020).

The ethyl acetate and the n-heptane extracts of *Helichrysum congolanum* were active against 4 *S. aureus* strains (MICs 250 to 1000 µg/mL, MBC 500 to 1000 and 1000 to 2000, MBC > 2000 µg/mL respectively). The other extracts were inactive on all tested strains. Although no antibacterial activity has been reported so far for *H. congolanum*, activities were shown for a number of other *Helichrysum* species, including *H. armenium* DC., *H.*

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pallasii Ledeb., *H. graveolens* (M.Bieb.) Sweet, *H. orientale* (L.) Vaill., *H. plicatum* subsp. *plicatum* and *H. petiolare* Hilliard & B.L.Burtt, against several bacterial strains, *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *P. aeruginosa*, *B. brevis*, *B. cereus*, *B. subtilis*, *S. aureus* (Akinyede et al., 2021; Lourens et al., 2011; Süzgeç-Selçuk & Birteksöz, 2011).

For *Justicia nyassana*, only the dichloromethane extract yielded an interesting activity against one of the tested *S. aureus* strains, LMG 8064 (MIC, 250 µg/mL; MBC, 1000 µg/mL). Interestingly, the dichloromethane, methanol and aqueous extracts of the Burundian *Justicia subsessilis* Oliv., currently reclassified as *Pogonospermum subsessile* (Oliv.) I.Darbysh. & Kiel., showed similar activities on the same *S. aureus* strains (MICs, 250 to 500 µg/mL; MBCs, 1000 µg/mL) (Ngezahayo et al., 2017). Given this reclassification, the chemical profiles of these probably closely related species would be interesting to compare.

The *Urtica massaica* ethyl acetate extract was active on *S. aureus* LMG 8064 (MIC, 250 µg/mL; MBC, 500 µg/mL). According to (Nahayo et al., 2008), total extracts and hydromethanolic fractions of *U. massaica* have interesting activities against enteropathogens (*Salmonella paratyphi*, *Shigella flexneri* and *E. coli*), which has been confirmed on *S. aureus* and *E. coli* (Allan et al., 2019). Of note, the methanolic extract and phenolic fractions of *U. dioica* L. leaves were active against *E. coli*, *S. enteridis*, *S. aureus*, *Listeria monocytogenes*, *Pseudomonas putida* and *B. cereus* (Assaf et al., 2020).

The *Senecio maranguensis* extracts were inactive on all tested bacterial strains, with MICs ≥ 2000 µg/mL.

TLC bioautography

For the most active plant (*Mikania natalensis)*, a bioautography test confirmed the presence of compounds active against MSSA in the extracts that showed bacterial growth inhibition (Figure 1).

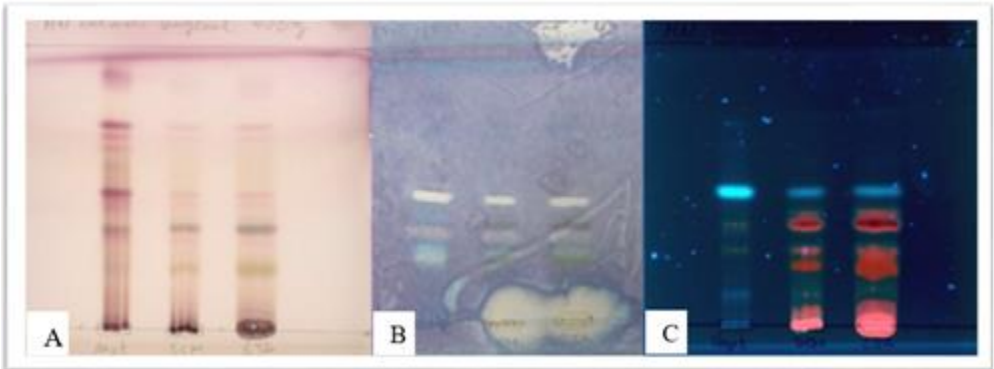

Figure 1 : TLC-Bioautography of n-heptane (track 1), dichloromethane (track 2) and ethyl acetate (track 3) extracts from the aerial parts of *Mikania natalensis* (successive extractions with increasing polarity solvents).

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Application of 40 µg for each extract (4 mg/mL, 10 µL). Mobile phase: n-hexane-ethyl acetate (80: 20, v/v). Derivatisation with anisaldehyde and sulfuric acid; the plate was observed under visible light (A) and 366 nm (C). For bioautography (B), a bacterial suspension of MSSA CNR 21248 was distributed on the plate that was then incubated at 37°C for 18-24 h and sprayed with MTT. The white spots indicate inhibition of bacterial growth (antibacterial activity) while the blue-purple background corresponds to bacterial growth (absence of antibacterial activity).

Figure 1 indicates the presence of 2 major antibacterial zones in the *M. natalensis* dichloromethane (MN/Di) and ethyl acetate (MN/Ac) extracts and one in the heptane extract. The MN/Di and MN/Ac extracts yield a very polar and practically non-migrating zone that is highly active. The bio-autography test also indicates a low-polarity active zone, present in the 3 extracts, which may correspond to a lower-solubility compound, with an activity probably difficult to detect in the microdilution method (MIC of the heptane extract > 1000 µg/mL).

Effect of M. natalensis extracts on MRSA strains

Table indicates that the combination of *M. natalensis* extracts with beta-lactams and aminoglycosides reduces the MICs of these antibiotics by a factor ranging from 2 to 16 (depending on the case), except for aqueous extracts, which counteracted the activity of these antibiotics. These results suggest that this plant is a potential resource for reducing MRSA resistance to certain important antibiotics.

Table 5 : Effects on MRSA of beta-lactams and aminoglycosides combined with *Mikania natalensis* extracts

Antibiotics and their combination with M. natalensis extracts (200 µg/mL)		**Minimum inhibitory concentrations (MIC, µg/ml)**		
		MRSA CNR 21249	MRSA CNR 21253	MRSA C100459
Beta-lactams	**Ampicillin alone**	**4**	**8**	**4**
	Ampicillin + MN/Hept	1	4	2
	Ampicillin + MN/Me	2	8	4
	Ampicillin + MN/Aq	Nd	Nd	Nd
	Oxacillin alone	**64**	**64**	**16**
	Oxacillin + MN/Hept	4	32	8
	Oxacillin + MN/Me	16	16	16
	Oxacillin + MN/Aq	Nd	Nd	Nd
	Penicillin G alone	**16**	**8**	**16**
	Penicillin G + MN/Hept	1	4	4

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	Penicillin G + MN/Me	1	4	4
	Penicillin G + MN/Aq	> 64	4	16
Aminoglycosides	**Gentamycin alone**	**16**	**0.25**	**16**
	Gentamycin + MN/Hept	4	≤ 0.06	2
	Gentamycin + MN/Me	8	0.12	8
	Streptomycin alone	**8**	**> 64**	**8**
	Streptomycin + MN/Hept	2	> 64	4
	Streptomycin + MN/Me	4	> 64	4
	Streptomycin + MN/Aq	> 64	> 64	4

MICs determined by microdilution tests, combining antibiotics (from 128 to 16 µg/mL, based on twice the MIC of each antibiotic acting alone) and *M. natalensis* extracts (200 µg/mL) over 18-24 h.
MRSA: methicillin-resistant S. aureus; MN/Hept: Heptane extract from *M. natalensis*; MN/Me: Methanol extract from *M. natalensis*; MN/Aq: Aqueous extract from *M. natalensis*; (successive extractions with increasing polarity solvents); Nd: not determined due to material limitations.

The most relevant antibiotic potentiation effects were observed with the heptane extract of *M. natalensis* (MN/He), which reduced the MICs of the tested antibiotics (ampicillin, oxacillin, penicillin G, gentamicin, and streptomycin) by 4–16-fold against the MRSA strain NCR 21249, by 2–4-fold, except for streptomycin, against the MRSA strain C 100459, and by 2–8-fold against the MRSA strain NCR 21253.

Determination of antioxidant activity

Antioxidant activities were quantified for the five methanolic extracts as IC_{50} values in µg/mL, representing the concentration of extract required to scavenge 50% of $DPPH^{\bullet}$ radicals, while IC_{50} QE values were calculated as quercetin equivalents to allow comparison with the reference antioxidant. *Helichrysum congolanum* and *Mikania natalensis* exhibited the highest antioxidant activities, with IC_{50} values of 148.1±5.7 and 187.2±0.2 µg/mL corresponding to 0.169±0.0065 and 0.1336±0.0001 QE/mL, respectively. Within the tested concentration range (125–300 µg/mL), the remaining three species (*Senecio maranguensis*, *Justicia nyassana*, and *Urtica massaica*) showed markedly lower activities, with IC_{50} values exceeding 300 µg/mL, corresponding to IC_{50} QE below 0.08 QE (Table 5 and Figure 3).

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Table 6 : Comparison of IC_{50} values for the methanolic extracts[(a)] of investigated plants

Plant	Part used	Estimated IC_{50} (µg/mL)	IC_{50} QE
Mikania natalensis	Aerial parts	187.2±0.2	0.1336±0.0001
Helichrysum congolanum	*Leaves*	148.1±5.7	0.169±0.0065
Justicia nyassana	*Leaves*	*> 300*	*< 0.08*
Senecio maranguensis	*Leaves*	*> 300*	*< 0.08*
Urtica massaica	*Aerial parts*	*> 300*	*< 0.08*
Quercetin	---	25.01±0.03	1

[(a)] Final step of successive extractions with increasing polarity organic solvents
[(b)] IC_{50} QE: IC_{50} values in Quercetin Equivalents.

This relatively limited activity may be attributed to the sequential extraction protocol employed, involving solvents of increasing polarities (Nzoyisubiziki et al., 2024). Thus, methanolic extracts, corresponding to the last step of this fractionated extraction, probably contain fewer antioxidants, as the less polar antioxidants (carotenoids, tocopherols, tocotrienols, flavonoid aglycones) have already been extracted by the preceding solvents.

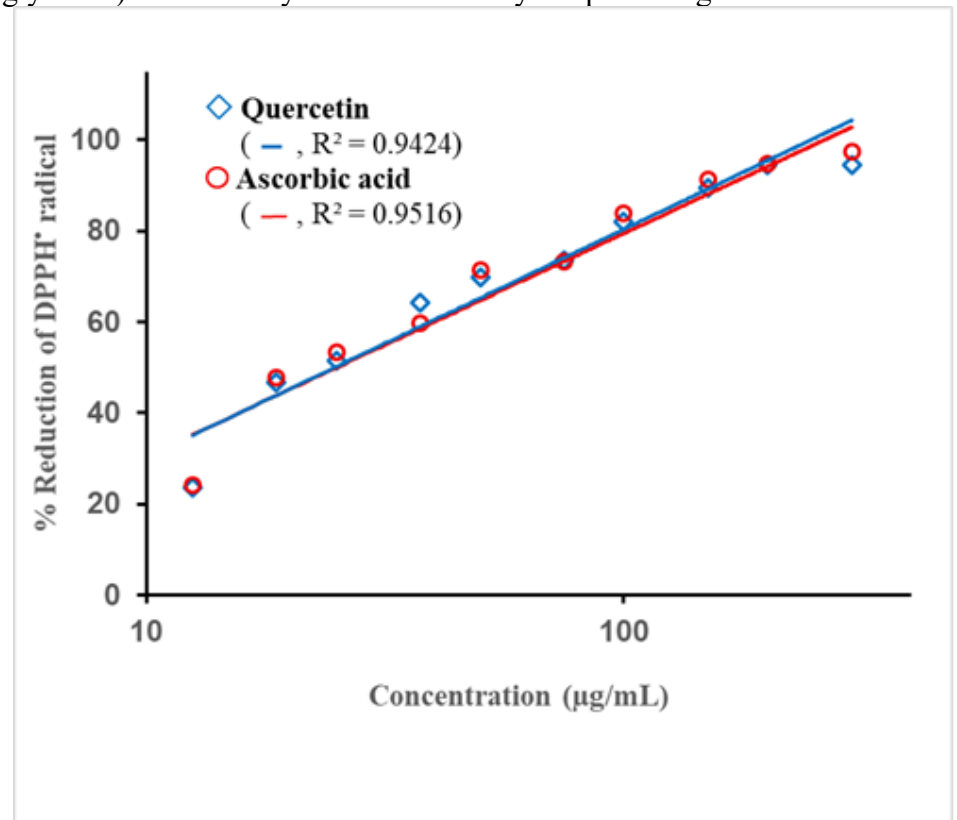

Figure 2 : DPPH• radical scavenging test using quercetin and ascorbic acid: DPPH• concentration, 0.04%; incubation time, 15 min in the dark; absorbance measured at 490 nm

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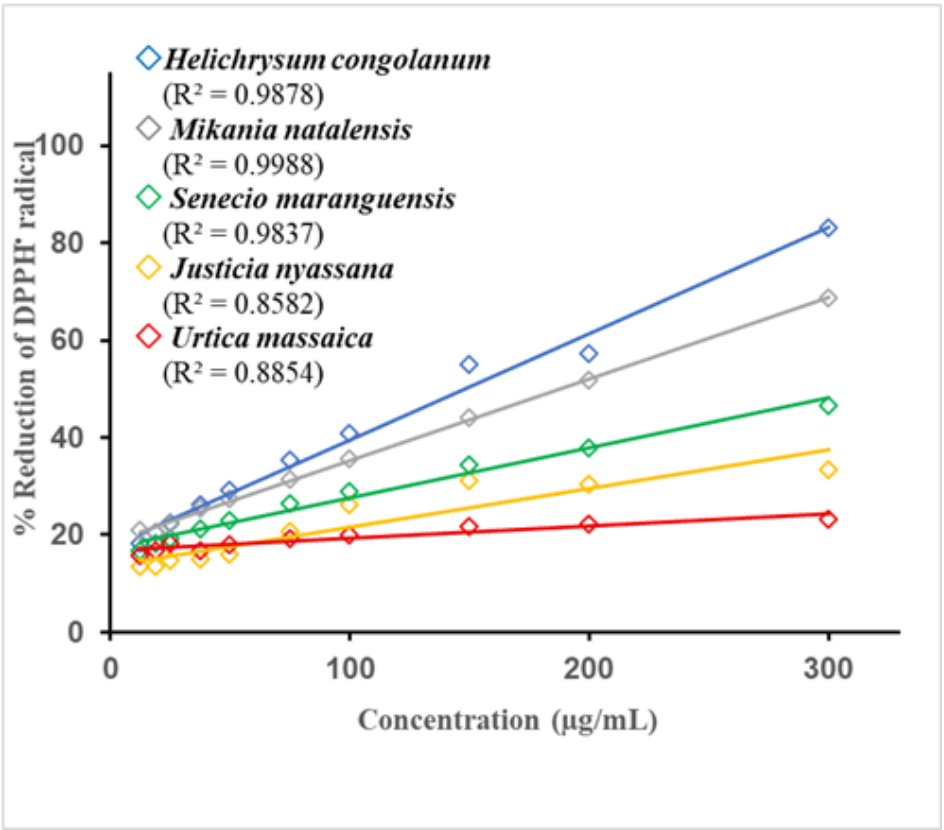

Figure 3 : DPPH• radical scavenging test using methanolic extracts from the 5 plants (Final step of successive extractions with increasing polarity organic solvents): DPPH• concentration, 0.04%; incubation time, 15 min in the dark; absorbance measured at 490 nm

To our best knowledge, the literature does not report studies on the antioxidant activities of these 5 plants, but only for other species belonging to the same genera as those studied. Regarding *Mikania*, antioxidant activities have been reported for the hydroethanolic extract of *M. glomerata* Spreng. and *M. laevigata* Sch. Bip ex Baker (Borghi et al., 2023), the ethanol extract of *M. cordata* (Burm.f.) B.L.Rob. (Ahmed, 2013; Khatun et al., 2020), the extracts of *M. scandens* (L.) Willd. (Khatun et al., 2020; Wijayaa et al., 2020) and *M. micrantha* Kunth (Khatun et al., 2020). Regarding *Helichrysum*, *Justicia* and *Urtica*, the methanol extract of the aerial parts of *H. chasmolycicum* P.H.Davis (Süzgeç-Selçuk & Birteksöz, 2011), the methanol and acetone extracts of *H. petiolare* Hilliard & B.L.Burtt (Akinyede et al., 2021), the hydroethanolic extracts of *J. spicigera* Schltdl. (Baqueiro-Peña & Guerrero-Beltrán, 2017) and the methanol extract of the whole plant *U. dioica* L. (Assaf et al., 2020; Pourmorad et al., 2006) effectively scavenged the DPPH• radical.

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Conclusions

The present study evaluated the antibacterial and antioxidant effects of crude successive extracts (heptane, dichloromethane, ethyl acetate, methanol, and water) obtained from five Burundian medicinal plants traditionally used to treat infectious diseases. Antibacterial activity was observed only against Gram-positive strains, particularly *S. aureus*. Four plants produced active extracts with MICs between 250 and 1000 µg/mL, while *S. maranguensis* extracts were inactive (MICs ≥ 2000 µg/mL). *Mikania natalensis* was the most active species, with dichloromethane and ethyl acetate extracts showing MICs of 250–500 µg/mL.

Interestingly, otherwise inactive extracts of *M. natalensis* (MICs ≥ 1000 µg/mL) potentiated β-lactams (ampicillin, oxacillin, penicillin G) and aminoglycosides (gentamicin, streptomycin) against 3 multidrug-resistant *S. aureus* strains, reducing antibiotic MICs by 2–16-fold. This synergy reinforces the potential of plant-derived compounds as adjuvants to conventional antibiotics in the fight against MRSA. Antioxidant activity varied among species, with *Helichrysum congolanum* and *Mikania natalensis* emerging as the most promising candidates.

However, the absence of conventional interaction metrics such as FICI or time-kill assays represents a limitation of this study. Future work will focus on bioassay-guided fractionation of the polar, non-migrating antibacterial zone detected by TLC-bioautography, followed by checkerboard (FICI) and time-kill assays to confirm and quantitatively characterize the observed antibiotic-potentiating effects. Given the known genotoxic and hepatotoxic pyrrolizidine alkaloids in the genus *Senecio*, caution is warranted, and quantitative evaluation of these compounds in *S. maranguensis* is essential before any translational application.

Overall, this work highlights the potential of Burundian medicinal plants as sources of bioactive compounds and antibiotic adjuvants, reinforcing their relevance in traditional medicine and in the global effort to combat antimicrobial resistance.

Conflict of Interest: The authors reported no conflict of interest.

Data Availability: All data are included in the content of the paper.

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