Heliyon 7 (2021) e06830

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

In vitro antimicrobial activity of *Millettia laurentii* De Wild and *Lophira alata* Banks ex C. F. Gaertn on selected foodborne pathogens associated to gastroenteritis

Hippolyte Tene Mouafo [a,*], Alex Dimitri Kamgain Tchuenchieu [a], Maxwell Wandji Nguedjo [a,b], Ferdinand Lanvin Ebouel Edoun [a,b], Boris Ronald Tonou Tchuente [a,b], Gabriel Nama Medoua [a]

[a] *Centre for Food and Nutrition Research, Institute of Medical Research and Medicinal Plants Studies, POBOX 13033, Yaoundé Cameroon*
[b] *Department of Biochemistry, Faculty of Sciences, University of Yaoundé 1, POBOX 812, Yaoundé Cameroon*

ARTICLE INFO

Keywords:
Millettia laurentii seeds
Lophira alata leaves
Extraction solvents
Antimicrobial activity
Foodborne pathogens

ABSTRACT

This study aimed at evaluating the antimicrobial potential of aqueous, ethanolic and methanolic extracts of two Cameroonian plants against selected foodborne pathogens. Bioactive compounds were extracted from *Millettia laurentii* De Wild seeds and *Lophira alata* Banks ex. C. F. Gaertn leaves using distilled water, ethanol and methanol as solvents. The extracts were tested against *Escherichia coli* O157, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Moraxella morganii*, *Salmonella enteritidis*, *Klebsiella pneumoniae* and *Listeria monocytogenes* using the microdilution method. The results showed that distilled water extracted a more important mass of phytochemical compounds (18.0–24.60%) compared to ethanol (4.80–5.0%) and methanol (4.20–4.60%). All the extracts exhibited significant antimicrobial activity with MIC values ranging from 5 to 20 μg/mL for *M. laurentii* seeds extracts and from 1.0 to 20 μg/mL for *L. alata* leaves extracts. The different plant extracts were ten times less active than gentamicin. The most active extracts were obtained using ethanol as solvent and *K. pneumoniae* was the most resistant pathogen to all extracts (MBC>20 μg/mL). *M. laurentii* extracts were bactericidal against *L. monocytogenes* and *P. mirabilis* while the reference antibiotic (gentamicin) was bacteriostatic against these pathogens. The results obtained from this study suggest the studied local plant materials as a source of antimicrobial compounds which can be valorized in the medical field as substitute of antibiotics for which many microorganisms have nowadays developed resistance mechanisms. Further studies need to be performed in order to characterize and identify these antimicrobial active molecules.

1. Introduction

Gastroenteritis can be defined as an inflammation of the stomach and gut walls derived from microbial infection and leading to diarrhoea, tenesmus, nausea, vomiting, combined with abdominal pain, or systemic symptoms such as fever vomiting, and sometime gross fecal blood loss (Al Jassas et al., 2018). According to Aziz and Bonnet (2008), approximately one person over ten contracts infectious gastroenteritis during his lifespan. Most of the affected people are from developing countries (70%) (WHO, 2015), and they are mainly children between 0 and 4 years (20%). Cameroon is also concerned as gastroenteritis disease is the second leading cause of death of children under 5 years (Black et al., 2010). The risk factors associated with gastroenteritis diseases are: age, immunosuppression, malnutrition, travel in endemic zone, exposition to precarious sanitary conditions, frequentation of hospital keeping services, consumption of contaminated food and water. Among these risk factors, consumption of food and water containing microorganisms are the main reported causes (WHO, 2015) as the frequency of travels and eating outside of the home are increasing nowadays (Okojie and Isah, 2014). Foodborne gastroenteritis diseases are a major public health concern and an important cause of morbidity and mortality worldwide. It mobilizes significant parts of health care resources, particularly in developing countries (WHO, 2015). Food contamination might occur at any stage of food production and can be the result of environmental contamination, including water and soil contamination. These microorganisms can be bacteria, viruses or parasites (Bruzzese et al., 2018). Bacterial gastroenteritis is mostly reported in developing countries (Giddings et al., 2016). The most incriminated bacteria are *Bacillus*

* Corresponding author.
E-mail address: hippolyte.tene@gmail.com (H.T. Mouafo).

https://doi.org/10.1016/j.heliyon.2021.e06830
Received 14 October 2020; Received in revised form 17 December 2020; Accepted 13 April 2021

cereus, *Staphylococcus aureus*, *Clostridium botulinum*, *Vibrio cholerae*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* and *Shigella* species and others which produce toxins that cause foodborne intoxinations (Malangu, 2016). It has been established that *Salmonella* spp. continued to be the most commonly detected cause in reported foodborne outbreaks (22.5% of total outbreaks (EFSA, 2015; Bari and Yeasmin, 2018). An unusual foodborne outbreak of gastroenteritis associated with contaminated turkey occurred at a catered company meal. Plasmid analysis and enterotoxin results supported the role of *Klebsiella pneumoniae* as the causative agent in this outbreak (Rennie et al., 1990). In India, strains of diarrheagenic *E. coli* (EPEC, STEC, EAEC, O 157 and EHEC) were notified as the most common agents of acute gastroenteritis (31%) (Shrivastava et al., 2017). According to cited reports, *Morganella morganii* and *Proteus mirabilis* were frequently isolated in patients with gastroenteritis (Muller, 1986). *Pseudomonas aeruginosa* mostly found in water is often involved in gastroenteritis diseases as it can develop in the gastrointestinal tract and cause infection (Huang et al., 2017).

As bacteria are generally the causative agents of gastroenteritis, antibiotic therapy is quite often used to treat diseases and prevent infection spreading (Bruzzesse et al., 2018). However, bacteria resistance to common antibiotics has become a serious matter. Some bacteria like *M. morganii*, *P. mirabilis* and *P. aeruginosa* were reported to possess chromosomally encoded β-lactamases belonging to the AmpC β-lactamase family and to produce extended spectrum β-lactamases responsible for their multiantibiotic resistance (Wilke et al., 2005; Bush and Fisher, 2011). Hence, to limit the spreading of multidrug resistant bacteria, researches are nowadays turned towards novel antibacterials. In this light, botanicals which constitute a good source of antimicrobial compounds appears as an alternative (Ngameni et al., 2013). In developing countries, due to low incomes and misused of antibiotics, the majority of the population now rely on plants or derived products for their treatment (WHO, 1993; Dongmo et al., 2015). Many plants have shown a good antimicrobial activity in the world as well as in the Cameroonian pharmacopoeia (Dongmo et al., 2015; Tchinda et al., 2017; Mostafa et al., 2017). However, the search for other antimicrobial plants from the local botanical resource is ongoing. In the present study, two Cameroonian plants *Millettia laurentii* De Wild and *Lophira alata* Banks ex C. F. Gaertn were investigated. Roots, leaves and barks of *M. laurentii* commercially called *Wengué* or *Awoung* in Cameroonian local languages were reported to have antitumoral, antiparasitic, antiviral and anti-inflammatory activities (Banzouzi et al., 2008). Barks of *L. alata* called *Azobé* (trade name) or *Abang* (*Nfon* language, Cameroon) were reported in traditional medicine to be active against fewer and gastroenteritis. Their leaves are used to the respiratory diseases (Biwolé et al., 2012). However, to the best of our knowledge, these two plants have not yet been tested against foodborne gastroenteritis microorganisms. Therefore, this study aimed at investigating the antimicrobial activity of different extracts from these plants against selected foodborne gastroenteritis bacteria.

2. Materials and methods

2.1. Vegetal materials

The vegetal materials used in this study were *M. laurentii* seeds and *L. alata* leaves. These plants were chosen because they are popular plants used in traditional medicine as judged by local healers and also because studies carried out on these plants (phytochemical screening) revealed their high contents in bioactive compounds such as polyphenols, tannins, flavonoids and alkaloids (Edoun et al., 2020). The plants were collected in April 2019 in the forest zone of the National School of Water and Forests of Mbalmayo (3°31′00″ N and 11°30′00 E), Centre Region of Cameroon. The database of the different plant species available at that Institute was used to facilitate the botanical identification. The collected plants were washed with distilled water, air-dried at 60 °C for 24 h (Memmert, Schwabach, Germany), ground (Moulinex, Lyon, France) and sieved. The powders of particle sizes lower than 1 mm were stored in a sterile airtight container until further use.

2.2. Microbial strains

Cultures of *Escherichia coli* O157, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Morganella morganii*, *Salmonella enteritidis*, *Klebsiella pneumoniae* and *Listeria monocytogenes*, associated to foodborne gastroenteritis outbreaks in the city of Ngaoundéré (Adamawa Region of Cameroon) were provided by the Laboratory of Food Microbiology and Biotechnology of the National School of Agro-industrial Sciences, University of Ngaoundéré (Cameroon). Prior utilization, the strains were sub cultured twice at 37 °C for 24 h in 10 mL of Brain Heart Infusion broth (LiofilChem, Via Scozia, Italy).

2.3. Chemicals

Methanol (99% of purity), ethanol (98% of purity), dimethyl sulfoxide (DMSO, 99% of purity), and gentamicin used in this study were purchased from Sigma-Aldrich Co., (Munich, Germany) while culture media namely Plate Count Agar (PCA) and Brain Heart Infusion (BHI) broth were purchased from LiofilChem (Via Scozia, Italy).

2.4. Extraction of active compounds

Three solvents reported as efficient for the extraction of bioactive compounds were used. They were methanol, ethanol and water. Fifty grams (50 g) of vegetal material was weighted and introduced into an Erlenmeyer. Then, 500 mL of solvent was added, and extraction process (maceration) carried out for 12 h at room temperature under agitation of 800 rpm using a magnetic hot plate stirrer (Lab-line Pyro-multi-Magnestir 1263-1, San Francisco, USA). The obtained extract was centrifuged at 3500 g for 20 min (Rotofix 32 A, Hettich Zentrifugen, Tuttlingen, Germany), the supernatant filtered (Whatman N°4) and oven-dried at 60 °C until a semisolid residue was obtained. The dried extract was weighted and stored at room temperature for analyses. Extraction yield was determined using the following formula:

$$Yield = \frac{Mass\ of\ extracted\ plant\ residues\ (g)}{Mass\ of\ plant\ raw\ sample\ (g)} \times 100$$

2.5. Antimicrobial activity

2.5.1. Inoculums preparation

The different strains were cultured for 16 h at 37 °C in 1 L of BHI broth. After incubation, the cells were collected by centrifugation (6500 g, 4 °C, 10 min), washed twice with sterile saline and resuspended in 5 mL of sterile saline. The suspensions were serial diluted, counted and the concentrations were adjusted to 5×10^5 CFU/mL using sterile saline (Cavalieri et al., 2005).

2.5.2. Preparation of antimicrobial solutions

For each plant extract, 0.2 g was aseptically weighted and introduced into a sterile tube containing 10 mL of a sterile solution of DMSO (1%, v/v). The plant extract was completely dissolved in DMSO solution by manual shaking. The solution obtained was used to prepare the different concentrations used in the analytical process.

2.5.3. Determination of minimum inhibitory concentration (MIC)

MIC is the lowest concentration of antibacterial agent that completely inhibits the visible bacterial growth. The macro dilution method of the American Society for Microbiology (Cavalieri et al., 2005) was used to determine the MIC of the different plant extracts with slight modifications. Briefly, 1.6 mL of sterile BHI broth was introduced into sterile test tubes. Then, 0.2 mL of inoculum suspension (5×10^5 CFU/mL) was

added into the tubes. The antimicrobial solution (0.2 mL) was then added and sterile BHI broth was used to adjust the final concentration to 0, 1.0, 5.0, 10.0, 15.0 and 20.0 μg/mL. The tubes were homogenized and incubated aerobically at 37 °C for 24 h. After incubation, MIC was determined by the unaided eye as the tube with the lowest concentration of antibacterial agent wherein no bacterial growth is observed. Each experiment was performed in triplicate. Gentamicin prepared in the same conditions as plant extracts was used as standard. The antibiotic was dissolved in DMSO 1% and the final tested concentrations were 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 μg/mL.

2.5.4. Determination of minimum bactericidal concentration (MBC)

MBC is the lowest concentration of antimicrobial agent that killed 99,99% of bacteria. The method of American Society for Microbiology with slight modifications was used (Cavalieri et al., 2005). A 100 μL volume of the preparation which did not show any growth after incubation during MIC assays was added into test tubes containing 1.9 mL of freshly prepared BHI broth. Experiments were performed in triplicate. The tubes were homogenized and incubated aerobically at 37 °C for 24 h. The test tubes with the lowest concentration of antimicrobial agent wherein no bacterial growth were observed were considered as MBC.

3. Results

3.1. Plants extraction yield

The extract yields gathered from the two plant materials while using methanol, ethanol and distilled water as solvents are presented in Table 1. As observed in Table 1, the highest yield (24.60%) for *M. laurentii* seeds was obtained after extraction with distilled water as solvent. Extraction with methanol and ethanol were less effective with a yield of 4.6 and 4.8%, respectively. A similar tendency was observed with *L. alata* leaves. Extraction yield with distilled water was 18.0% while it was just 4.2 and 5.0% with methanol and ethanol, respectively.

3.2. Antimicrobial activity of plant extracts

3.2.1. Minimum inhibitory concentration (MIC)

The MIC of the different plant extracts are presented in Table 2. All extracts were active against the different pathogens with MIC values ranging from 5 to 20 μg/mL for *M. laurentii* seeds and from 1.0 to 20 μg/mL for *L. alata* leaves.

Regarding *M. laurentii* seeds, the highest antagonistic activity was recorded against *L. monocytogenes* whatever the extraction solvent used. Globally, aqueous extract was less active against all the tested microorganisms compared to ethanolic or methanolic one. Ethanolic extracts appeared more active than methanolic extracts against some pathogens like *E. coli* O157, *M. morganii* and *P. mirabilis*. However, both extracts exhibited the same MIC values against the rest of microorganisms tested. Amongst the studied strains, *K. pneumoniae* with MIC values of 20 μg/mL independent of the extraction solvent used, was the most resistant strain.

Concerning *L. alata* leaves, ethanolic extract displayed the highest activity against *S. enteritidis*, *M. morganii*, *P. mirabilis* and *B. cereus* with MIC of 5.0, 1.0, 1.0 and 1.0 μg/mL, respectively. Similar inhibitory activities of methanolic and ethanolic extracts were noticed on *L. monocytogenes*, *S. aureus*, *K. pneumoniae*, *E. coli* O157 and *P. aeruginosa*. Aqueous extract with MIC values ranging from 10 to 20 μg/mL was less active against all the strains tested. *K. pneumoniae* also appeared as the most resistant strain (MIC values of 20 μg/mL) while *L. monocytogenes* was the most sensitive strain.

A global comparison of the inhibitory activity tended to show that extracts deriving from *L. alata* leaves were more active than those from *M. laurentii* seeds. However, the exhibited activity was far lower than the one observed with the reference antibiotic, namely gentamicin, which exhibited MIC values ranging from 0.1 to 5.0 μg/mL.

3.2.2. Minimum bactericidal concentration (MBC)

Table 3 summarizes the results of MBC of the different plant extracts against some selected foodborne pathogens. The MBC values obtained range from 5 μg/mL to more than 20 μg/mL for *L. alata* leaves extracts and from 10 to more than 20 μg/mL for *M. laurentii* seeds extracts. Ethanolic and methanolic extracts have generally showed the lowest MBC values independently of the plant material or tested strain in comparison to aqueous extracts.

For *M. laurentii* seeds, the most sensitive strain independent of the extraction solvent used was *L. monocytogenes* while *P. aeruginosa*, *S. enteritidis*, *K. pneumoniae* and *S. aureus* with MBC of 20 μg/mL were the most resistant strains. This was also the case with *L. alata* leaves, for which the most sensitive strain was *L. monocytogenes* with MBC values of 15.0, 5.0 and 5.0 μg/mL for aqueous, ethanolic and methanolic extracts, respectively. *K. pneumoniae* was the most resistant strain with MBC value >20 μg/mL independently of the extraction solvent. Considering the low MBC values obtained with gentamicin, this reference antibiotic showed a bactericidal activity that was quite higher compared to all the tested plant extracts.

3.2.3. MBC/MIC ratio

In order to define the bactericidal or bacteriostatic status of the different plant extracts, MBC/MIC ratio were calculated (Table 4). The values obtained ranged from 1.3 to 3 for *M. laurentii* seed extracts while for *L. alata* leaves extracts, it ranges from 1 to 5. The highest MBC/MIC ratio were recorded with ethanolic extracts of both *M. laurentii* seeds and *L. alata* leaves.

4. Discussion

M. laurentii and *L. alata* are both plants used as traditional medicine for the management of many diseases in Africa including those derived from foodborne pathogens knowing as gastroenteritis. They are mainly used as decoctions with water as solvent. Giving that some antimicrobial compounds presented in plants are mostly insoluble in water, it therefore appeared interesting to check the antimicrobial activity of other extracts from these plants. In this study, ethanol and methanol were used as solvents to extract antimicrobial compounds presented in *M. laurentii* seeds and *L. alata* leaves, this in comparison with distilled water. Higher extraction yields were recorded with distilled water as solvent, meaning that water extracts the most important mass compounds present in the plant material. This could be attributed to the polarity of solvents and thus, to the important proportions of water soluble compounds present in plant. Padalia and Chanda (2015) also highlighted in their studies on *Tagetes erecta* flowers, the superiority of water to extract an important mass of phytoconstituents compared to methanol and other organic solvents.

High yields of phytoconstituents obtained does not necessarily imply a high antimicrobial activity (Padalia and Chanda, 2015). The tested extracts showed a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria but ethanolic and methanolic extracts were more active than aqueous extracts. This difference could be explained by the fact that organic solvent like methanol and ethanol can easily pass through the cell membrane and extracted insoluble secondary

Table 1. Extract yields (%) obtained from the two plants with different solvents.

Scientific name of plants	Local name	Part of plant used	Solvents	Yields (%)
Millettia laurentii De Wild	Awoung	Seeds	Methanol	4.60
			Ethanol	4.80
			Distilled water	24.60
Lophira alata Banks ex C. F. Gaertn	Abong	Leaves	Methanol	4.20
			Ethanol	5.00
			Distilled water	18.00

Table 2. Minimum Inhibitory Concentration (μg/mL) of different plants extracts against pathogens.

Pathogens	Plants						Control
	M. laurentii seeds			*L. alata* leaves			
	AEWS	EEWS	MEWS	AEAL	EEAL	MEAL	Gentamicin
P. aeruginosa	20.0	10.0	10.0	20.0	10.0	10.0	2.0
E. coli O157	20.0	5.0	10.0	15.0	5.0	5.0	1.0
S. enteritidis	15.0	10.0	10.0	15.0	5.0	10.0	0.2
K. pneumoniae	20.0	20.0	20.0	20.0	20.0	20.0	5.0
M. morganii	15.0	5.0	10.0	15.0	1.0	5.0	0.5
P. mirabilis	10.0	5.0	10.0	10.0	1.0	5.0	0.2
S. aureus	20.0	15.0	15.0	15.0	10.0	10.0	1.0
B. cereus	15.0	5.0	5.0	10.0	1.0	5.0	0.5
L. monocytogenes	10.0	5.0	5.0	10.0	1.0	1.0	0.1

AEWS = Aqueous extract of *M. laurentii* seeds; EEWS = Ethanolic extract of *M. laurentii* seeds; MEWS = Methanolic extract of *M. laurentii* seeds; AEAL = Aqueous extract of *L. alata* leaves; EEAL = Ethanolic extract of *L. alata* leaves; MEAL = Methanolic extract of *L. alata* leaves.

Table 3. Minimum Bactericidal Concentration (μg/mL) of different plants extracts against pathogens.

Pathogens	Plants						Control
	M. laurentii seeds			*L. alata* leaves			
	AEWS	EEWS	MEWS	AEAL	EEAL	MEAL	Gentamicin
P. aeruginosa	>20.0	20.0	20.0	>20.0	15.0	20.0	5.0
E. coli O157	>20.0	15.0	15.0	20.0	10.0	10.0	2.0
S. enteritidis	20.0	20.0	15.0	20.0	10.0	10.0	0.5
K. pneumoniae	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	15.0
M. morganii	20.0	10.0	15.0	20.0	5.0	15.0	1.0
P. mirabilis	15.0	15.0	15.0	15.0	5.0	10.0	1.0
S. aureus	>20.0	20.0	20.0	20.0	10.0	15.0	5.0
B. cereus	20.0	10.0	10.0	15.0	5.0	10.0	1.0
L. monocytogenes	15.0	10.0	10.0	15.0	5.0	5.0	0.5

AEWS = Aqueous extract of *M. laurentii* seeds; EEWS = Ethanolic extract of *M. laurentii* seeds; MEWS = Methanolic extract of *M. laurentii* seeds; AEAL = Aqueous extract of *L. alata* leaves; EEAL = Ethanolic extract of *L. alata* leaves; MEAL = Methanolic extract of *L. alata* leaves.

Table 4. MBC/MIC ratio of different plants extracts against pathogens.

Pathogens	Plants						Control
	M. laurentii seeds			*L. alata* leaves			
	AEMS	EEMS	MEMS	AELL	EELL	MELL	Gentamicin
P. aeruginosa	/	2.0	2.0	/	1.5	2.0	2.5
E. coli O157	/	3.0	1.5	1.3	2.0	2.0	2.0
S. enteritidis	1.3	2.0	1.5	1.3	2.0	1.0	2.5
K. pneumoniae	/	/	/	/	/	/	3.0
M. morganii	1.3	2.0	1.5	1.3	5.0	3.0	2.0
P. mirabilis	1.5	3.0	1.5	1.5	5.0	2.0	5.0
S. aureus	/	1.3	1.3	1.3	1.0	1.5	5.0
B. cereus	1.3	2.0	2.0	1.5	5.0	2.0	2.0
L. monocytogenes	1.5	2.0	2.0	1.5	5.0	5.0	5.0

AEWS = Aqueous extract of *M. laurentii* seeds; EEWS = Ethanolic extract of *M. laurentii* seeds; MEWS = Methanolic extract of *M. laurentii* seeds; AEAL = Aqueous extract of *L. alata* leaves; EEAL = Ethanolic extract of *L. alata* leaves; MEAL = Methanolic extract of *L. alata* leaves./ = not applicable.

metabolites present in the plants like flavonoids, tannins, terpenoids, phenolic compounds and alkaloids which are potentially endowed with antibacterial properties (Onivogui et al., 2015; Al Farraj et al., 2020). In previous studies carried out on *M. laurentii* seeds and *L. alata* leaves, it was demonstrated that these plants contained high amounts of flavonoids, polyphenols, tannins and alkaloids (Edoun et al., 2020). The difference of concentration of these compounds varying with the solvent (Nair et al., 2006) may therefore explain the different antibacterial activity observed later. Some of these bioactive secondary metabolites are known to interact with proteins located in the bacterial cell membrane and mitochondria, disturb their structures and change their permeability, thus leading to cell death through its disruption (Tiwari et al., 2009). Their inhibitory effect is also characterized by the ability of phenolic compounds of the different plant extracts to interact with microbial enzymes necessary for amino acids biosynthesis (Tiwari et al., 2009). The higher antimicrobial activity observed with ethanolic plant

extracts compared to methanolic extracts independent of the plant had already been reported by Al Farraj et al. (2020) with extracts from *Dipcadi viride*.

From the tested strains, *K. pneumoniae* was the most resistant to all extracts independent of solvents used. This could be due to the fact that, besides the solvent polarity which lead to extraction of various amount of bioactive compounds and thus to different antibacterial activity, the bacterial strain involved also plays a significant role as each bacterium responds differently to bioactive compounds (Chandra et al., 2017; Khameneh et al., 2019). In a study performed by Padalia and Chanda (2015), the authors highlighted that, amongst the tested bacteria, *K. pneumonia* was more sensitive to extracts derived from non-polar solvents such as hexane compared to those derived from polar solvents.

The methanolic extracts of *L. alata* leaves with their MIC values of 10.0, 5.0 and 20.0 μg/mL against *P. aeruginosa E. coli* O157 and *K. pneumoniae* respectively, were more active than those reported in the literature by Tchinda et al. (2017) with the methanolic extracts of the leaves of a Cameroonian medicinal plant named *Alchornea laxiflora*. They noticed MIC of 256 μg/mL against *E. coli* ATCC8739 and *Klebsiella pneumoniae* ATCC11296, and MIC of 512 μg/mL against *Pseudomonas aeruginosa* PA01. This difference could be attributed to the profile of bioactive compounds which varies from one plant to another. The antimicrobial resistance mechanism which varies from a strain to another (Anderson, 2005; Andersson et al., 2016; Chandra et al., 2017; Khameneh et al., 2019) could also explain the difference of antimicrobial activity observed.

Dongmo et al. (2015) reported with methanolic extracts of *Moringa oleifera* seeds, MIC values of 5.0 mg/mL against *S. typhi* and *B. cereus* and 2.5 mg/mL against *S. paratyphi* and *E. coli*. These MIC values are quite higher compared to those observed in this study with methanolic extract *M. laurentii* seeds. *M. oleifera* seeds are used in traditional medicine to treat patients suffering of diarrhoea due to microorganisms (Fahey, 2005). Therefore, *M. laurentii* seeds extracts with its activity against gastroenteritis-causing bacteria, represent a promising source of antibacterial biomolecules which can be used to treat diarrhoea.

According to the literature cited reports, a plant extract is considered showing significant antimicrobial activity against a specific microorganism when its MIC value against this microbial strain is below 100 μg/mL (Kuete, 2010; Kuete and Efferth, 2010). When its MIC is between 100 and 625 μg/mL, it activity is considered moderate and when its MIC is higher than 625 μg/mL, it activity is considered weak (Kuete, 2010; Kuete and Efferth, 2010). Hence, the antimicrobial activity of *M. laurentii* seeds extracts as well as those of *L. alata* leaves extracts could be considered significant against all the microorganisms tested in this study.

The bactericidal nature of an antimicrobial compound can also be appreciated through the MBC/MIC ratio (Oussou et al., 2008). When the MBC/MIC ratio of an antimicrobial compound against a specific strain is $\leq$4, that compound is considered as microbiocidal against the tested strain (Oussou et al., 2008; Teke et al., 2011). On this basis, methanolic and ethanolic extracts *M. laurentii* seeds could be considered bactericidal against all the tested strains as their MBC/MIC ratio were between 1.3 and 3 with an exception to *K. pneumoniae*.

On the other side, an MBC/MIC ratio above 4 were obtained with *L. alata* leaves ethanolic extract against *M. morganii*, *P. mirabilis*, *B. cereus* and *L. monocytogenes*. Meaning that ethanolic extract was bacteriostatic against these pathogens. Gentamicin also appeared as having a bacteriostatic effect against these pathogens. In contrast, methanolic extract of *L. alata* leaves was bactericidal against the tested pathogens except *L. monocytogenes*. Although aqueous extract of *L. alata* leaves presented MIC and MBC values lower than that of ethanolic extract, they were bactericidal against all the tested strains excepted *P. aeruginosa* for which MBC/MIC ratio could not be estimated.

5. Conclusion

The present study demonstrated the significant and broad spectrum of antimicrobial activity of aqueous, methanolic and ethanolic extracts of *M. laurentii* seeds and *L. alata* leaves against several Gram-positive and Gram-negative pathogens commonly incriminated in human health problems. This study highlights the antimicrobial potential of local plant materials which can be valorize in food industries as biopreservative as well as in the medical field as substitute of antibiotics. For this, further studies on the structural characterization and the antimicrobial mechanism of these extracts need to be performed.

Declarations

Author contribution statement

Hippolyte Tene Mouafo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Alex Dimitri Kamgain Tchuenchieu: Conceived and designed the experiments; Wrote the paper.

Maxwell Wandji Nguedjo, Ferdinand Lanvin Ebouel Edoun: Performed the experiments; Wrote the paper.

Boris Ronald Tonou Tchuente: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Gabriel Nama Medoua: Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the commercial, public, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors thank the collaboration of the National School of Water and Forest (NSWF) of Cameroon, especially Mr. Woincham Tohnain, for the help provided in identifying the plants.

References

Al Farraj, D.A., Abdel Gawwad, M.R., Mehmood, A., Alsalme, A., Darwish, N.M., Al-Zaqri, N., Warad, I., 2020. In-vitro antimicrobial activities of organic solvent extracts obtained from *Dipcadi viride* (L.) Moench. J. King Saud Univ. Sci. 32 (2020), 1965–1968.

Al Jassas, B., Khayat, M., Alzahrani, H., Asali, A., Alsohaimi, S., Al Harbi, H., Al Qadi, M., Al Qassim, M., Mutahar, A., Mahbub, M., 2018. Gastroenteritis in adults. Int. J. Commun. Med. Publ. Health. 5 (11), 4959–4964.

Anderson, D.I., 2005. The ways in which bacteria resist antibiotics. Int. J. Risk Saf. Med. 17, 111–116.

Andersson, D.I., Hughes, D., Kubicek-Sutherland, J.Z., 2016. Mechanisms and consequences of bacterial resistance to antimicrobial peptides. Drug Resist. Updates 26, 43–57.

Aziz, K., Bonnet, D., 2008. Hepato-gastro-enterology, first ed. Elsevier Masson, Paris, France.

Banzouzi, J.T., Prost, A., Rajemiarimiraho, M., Ongoka, P., 2008. Traditional uses of African *Milletia* species (Fabaceae). Int. J. Bot. 4 (4), 406–420.

Bari, M.L., Yeasmin, S., 2018. Foodborne diseases and responsible agents. In: Grumezescu, A. (Ed.), Food Safety and Preservation. Elsevier Inc., pp. 195–229

Biwolé, A.B., Bourland, N., Daïnou, K., Doucet, J.L., 2012. Definition of the ecological profile of the *Azobe, Lophira alata*, an African woody species of great importance: literature review and perspectives for future research. Biotechnol. Agron. Soc. Environ. 16 (2), 217–228.

Black, E.R., Cousens, S., Johnson, H.L., Lawn, J.E., Rudan, I., Bassani, D.G., Jha, P., Campbell, H., Walker, C.F., Cibulskis, R., Eisele, T., Liu, L., Mathers, C., 2010. Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet 375, 1969–1987.

Bruzzesse, E., Giannattasio, A., Guarino, A., 2018. Antibiotic treatment of acute gastroenteritis in children. F1000Research. 7, 193.

Bush, K., Fisher, J.F., 2011. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamase from Gram-negative bacteria. Annu. Rev. Microbiol. 65, 455–478.

Cavalieri, S.J., Rankin, I.D., Harbeck, R.J., Sautter, R.L., McCarter, Y.S., Sharp, S.E., Ortez, J.H., Spiegel, C.A., 2005. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology, USA.

Chandra, H., Bishnoi, P., Yadav, A., Patni, B., Mishra, A.P., Nautiyal, A.R., 2017. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials—a review. Plants 6, 16.

Dongmo, N.A., Nganso, D.Y.O., Nkwengoua, T.E., Boda, M., Voundi, O.S., Etoa, F.X., Nyasse, S., 2015. In-vitro testing of extracts and fractions from two Cameroonian medicinal plants on bacteria gastroenteritis. Am. J. Phytomed. Clin. Ther. 3 (9), 575–588.

Edoun, E.F.L., Tchuente, T.B.R., Dibacto, K.E.R., Mouafo, T.H., Tchuenchieu, K.A.D., Ndzana, A.A.C., Medoua, N.G., 2020. Phytochemical screening and antioxidant potential of aqueous extracts of *Millettia laurenti, Lophira alata* and *Milicia excelsa*, commonly used in the Cameroonian pharmacopoeia. Eur. J. Med. Plants 31 (11), 11–23.

European Food Safety Authority (EFSA), 2015. European Centre for Disease Prevention and Control, 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J 13 (1), 3991.

Fahey, J.W., 2005. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for Life J. 157, 1–15.

Giddings, S.L., Stevens, A.M., Leung, D.T., 2016. Traveler's diarrhea. Med. Clin. 100 (2), 317–330.

Huang, C.H., Lin, T.Y., Chiu, C.H., 2017. *Pseudomonas aeruginosa*-associated diarrheal diseases in children. Pediatr. Infect. Dis. J. 36 (12), 1119–1123.

Khameneh, B., Iranshahy, M., Soheili, V., Bazzaz, B.S.F., 2019. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob. Resist. Infect. Contr. 8, 118.

Kuete, V., 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med. 76, 1479–1491.

Kuete, V., Efferth, T., 2010. Cameroonian medicinal plants: pharmacology and derived natural products. Front. Pharmacol. 1, 123.

Malangu, N., 2016. Risk factors and outcomes of food poisoning in Africa. In: Makun, H.A. (Ed.), Significance, Prevention and Control of Food Diseases. Intech Open, pp. 59–97.

Mostafa, A.A., Al-Askar, A.A., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N., Bakri, M.M., 2017. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J. Biol. Sci. 25 (2018), 361–366.

Muller, H.E., 1986. Occurrence of pathogenic role of Morganella – Proteus – Providencia group bacteria in human feces. J. Clin. Microbiol. 23 (2), 404–405.

Nair, R., Shah, A., Baluja, S., Chanda, S., 2006. Synthesis and antibacterial activity of some Schiff base complexes. J. Serb. Chem. Soc. 71 (7), 733–744.

Ngameni, B., Fotso, G.W., Kamga, J., Ambassa, P., Abdou, T., Fankam, A.G., Voukeng, I.K., Ngadjui, B.T., Abegaz, B.M., Kuete, V., 2013. 9-Flavonoids and related compounds from the medicinal plants of Africa. In: Kuete, V. (Ed.), Medicinal Plant Research in Africa: Pharmacology and Chemistry. Elsevier, Oxford, pp. 301–350.

Okojie, P.W., Isah, E.C., 2014. Sanitary conditions of food vending sites and food handling practices of street food vendors in Benin city, Nigeria: implication for food hygiene and safety. J. Environ. Publ. Health 2014, 1–6.

Onivogui, G., Letsididi, R., Diaby, M., Wang, L., Song, Y., 2015. Influence of extraction solvents on antioxidant and antimicrobial activities of the pulp and seed of *Anisophyllea laurina* R. Br. ex Sabine fruits. Asian. Pac. J. Trop. Biomed. 6 (1), 20–25.

Oussou, K.R., Coffi, K., Nathalie, G.S., Gerard, K., Mireille, D., Yao, T.N., Gille, F., Jean-Claude, C.H., 2008. Activités antibactériennes des huiles essentielles de trois plantes aromatiques de Côte d'Ivoire. C. R. Chim. 7, 1081–1086.

Padalia, H., Chanda, S., 2015. Antimicrobial efficacy of different solvent extracts of *Tagetes erecta* L. flower, alone and in combination with antibiotics. Appl. Microbiol. 1 (1), 1–10.

Rennie, R., Anderson, C.M., Wensley, B.G., Albritton, W.L., Mahony, D.E., 1990. *Klebsiella pneumoniae* gastroenteritis masked by *Clostridium perfringens*. J. Clin. Microbiol. 28 (2), 216–219.

Shrivastava, A.K., Kumar, S., Mohakud, N.K., Suar, M., Sahu, P.S., 2017. Multiple etiologies of infectious diarrhea and concurrent infections in a pediatric outpatient-based screening study in Odisha, India. Gut Pathog. 11 (9), 1–16.

Tchinda, C.F., Voukeng, I.K., Beng, V.P., Kuete, V., 2017. Antibacterial activities of the methanol extracts of *Albizia adianthifolia, Alchornea laxiflora, Laportea ovalifolia* and three other Cameroonian plants against multi-drug resistant Gram-negative bacteria. Saudi J. Biol. Sci. 24 (2017), 950–955.

Teke, G.N., Kuiate, J.R., Kuete, V., Teponno, R.B., Tapondjou, L.A., Tane, P., Giacinti, G., Vilarem, G., 2011. Bio guided isolation of potential antimicrobial and antioxidant agents from the stem bark of *Trilepisium madagascariense*. South Afr. J. Bot. 77, 319–327.

Tiwari, B.K., Valdramidi, V.P., O'Donnell, C.P., Muthukumarappan, K., Bourke, P., Cullen, P.J., 2009. Application of natural antimicrobials for food preservation. J. Agric. Food Chem. 57, 5987–6000.

Wilke, M.S., Lovering, A.L., Strynadka, N.C.J., 2005. β-Lactam antibiotic resistance: a current structural perspective. Curr. Opin. Microbiol. 8, 525–533.

World Health Organization (WHO), 1993. Summary of WHO guidelines for assessment of herbal medicines. HerbalGram 28, 13–14.

World Health Organization (WHO), 2015. Estimates of the Global burden of Foodborne Diseases. Foodborne Diseases burden Epidemiology Reference Group 2007–2015. World Health Organization, Geneva.