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

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**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 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 (Bruzesse et al., 2018). Bacterial gastroenteritis is mostly reported in developing countries (Giddings et al., 2016). The most incriminated bacteria are *Bacillus...
cereus, Staphylococcus aureus, Clostridium botulinum, Vibrio cholerae, Escherichia coli, Listeria monocytogenes, Salmonella and Shigella species and others which produce toxins that cause foodborne intoxications (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, as the most common agents of acute gastroenteritis (31%) (Shrivastava et al., 2017). 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 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. 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
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

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 | 5.0 | 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 | 15.0 | 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 |
| AESM | 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 | 3.0 |
| 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; EESM = Ethanolic extract of M. laurentii seeds; MEWS = Methanolic extract of M. laurentii seeds; AEAL = Aqueous extract of L. alata leaves; EEL = Ethanolic extract of L. alata leaves; MEAL = Methanolic extract of L. alata leaves./ = not applicable.
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 bacteria 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 Tchienda et al. (2017) with the methanolic extracts of the leaves of a Cameroonian medicinal plant named Alchornea laziflora. 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 St. typhi and B. ceraus 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 ≤4, that compound is considered as microbicidal 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. cerasus 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.
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