*Original Research Article*

**Investigating the antimicrobial potentials of *Cajanus cajan*(L.) Millsp. leaf extract On Healthcare Environment’s Multidrug-Resistant Bacteria**

**ABSTRACT**

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| **Background and objective:** Healthcare-associated infections (HAIs)-related clinical manifestations develop in patients who undergo infections caused by the healthcare setting. These infections are frequent causes of morbidity and mortality in both developed and developing countries. To promote medicinal plants as an alternative therapeutic to current conventional antibacterial agents, which are increasingly tolerated by bacteria, the present investigation assessed the antibacterial potentials of the hydroethanolic extract recovered from dried leaves of *Cajanus cajan* (L.) Millsp., on multidrug-resistant bacteria isolated from a healthcare facility environment. **Methods:** For this purpose, multidrug-resistant bacteria were isolated from specimens collected from the “Université des Montagnes” Teaching Hospital environment, where local bacterial flora were previously found to express high rates of resistance to conventional antibacterial agents and disinfectants. The antibacterial potential was investigated through the determination of the minimal inhibitory and bactericidal concentrations of the extract from which a few groups of secondary metabolites were screened. **Results:** Qualitative phytochemical screening revealed the presence of flavonoids, polyphenols, quinones, tannins and terpenes in the extract used. On a pool of 64 multidrug-resistant bacteria isolates (Gram-positive rods: 23; Gram-positive cocci: 20; Gram-negative rods: 21), the minimal inhibitory and bactericidal concentrations of the hydroethanolic extract from dried leaves of *Cajanus cajan* (L.) Millsp. ranged from 1.465 through 93.75 mg/mL, and from 6.25 through 175 mg/mL, respectively. Gram-positive rods exhibited the highest tolerance (MIC = 69.4185±17.4405 mg/mL; MBC = 116.8478±35.677 mg/mL), while Gram-positive cocci were most susceptible (MIC = 3.2663±1.6854 mg/mL; MBC = 12.0701±5.7882 mg/mL). Amongst the Gram-negative rods, higher resistance was observed with *Pseudomonas* (MIC = 43.2292±3.0725 mg/mL; MBC = 73.9583±11.2962 mg/mL), and *Enterobacteriaceae* (MIC = 12.2342±3.6743 mg/mL; MBC = 22.1153±6.3016 mg/mL). Eventually, this extract expressed a bactericidal potential on 78.125% of subjected isolates. **Conclusion:** The antibacterial assays revealed a significant antibacterial activity against MDR-gram-positive cocci and gram-negative rods, which is due to bioactive compounds present in the plant as revealed by the phytochemical screening. Hence, the current findings indicate that this extract could effectively serve in controlling antibiotic-resistant bacteria, which are a threat in clinical settings and to public health at large. |

*Keywords:* Antibacterial potential, *Cajanus cajan*, Minimal Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (MBC)

, Multidrug-resistant bacteria

1. INTRODUCTION

The prevalence of drug-resistant microbial diseases acquired in healthcare facilities is high and deserves special attention. Drug-resistant bacterial infection significantly increases the mortality rate, hospitalisation time, and treatment cost, which increases with age (Wei et al.,2023; Gajic et al.,2023). Contracted during healthcare procedures and formerly referred to as hospital-acquired, healthcare-associated infections (HAIs)-related clinical manifestations develop in patients who undergo infections caused by the healthcare setting (WHO, 2010; Rambaud, 2008). HAIs can occur in various wards during treatment; they are most often associated with hospitalisation in intensive care units (ICUs) (Dadi et al.,2021; Szabó et al.,2022). These infections are frequent causes of morbidity and mortality in both developed and developing countries. The global picture estimates that about 5% of hospitalised patients develop HAIs every year (Nouetchognou *et al*., 2016). Observance of specific guidance aiming at controlling the phenomenon to be effective in reducing its incidence in all settings, though at varied ranges (Nouetchognou *et al*, 2016), in connection with available human, technical and financial resources. In contexts characterised by drug-resistant etiologies, they are regarded as serious threats to all healthcare systems (Antimicrobial Resistance Collaborators, 2022). With increased resistance to disinfectants (Rozman *et al*, 2021), preventing HAIs is therefore seriously threatened, especially amongst populations that cannot afford conventional amenities. The surveys conducted in 2010 reported 20.74% of HAIs in Cameroon (Nouetchognou *et al*, 2016).

Antimicrobial resistance (AMR) has been prioritised by the World Health Organisation (WHO) as one of the top ten global public health threats facing humanity (Walsh et al.,2023; Salam et al.,2023). To address antimicrobial resistance as a global health issue, investigations through alternatives to drugs that are no longer effective in controlling microbial infections was encouraged by the WHO, and several studies are currently conducted in the potential of some plants and plant extracts, known for their antimicrobial and/or their therapeutic effects on microorganisms (Porras *et al*., 2021; Abass *et al*., 2022). All aim at identifying alternatives for disease prevention and control, primarily amongst the human population in low-income communities worldwide (Ouro-Djeri *et al*., 2022). In this vein, pharmacological studies have demonstrated that some plants from the *Fabaceae* family were potent in controlling microbial populations (Hannani *et al*., 2014). In this family, *Cajanus cajan* or “pigeon pea” is empirically used by some communities as traditional medicine to treat several microbial infection conditions (Okigbo and Omodamiro, 2007; Pal *et al*., 2011). Several research studies have reported confirming the antibacterial properties of *Cajanus cajan* (Okigbo and Omodamiro, 2007; Kong *et al*., 2010; Nwachukwu and Uzoeto, 2010; Oyewole *et al*., 2010; Pal *et al*., 2011; Pratima and Mathad, 2011; Ahomadegbe *et al*., 2018; Gargi *et al*., 2022; Yilwa *et al*., 2023). However, its significance in the healthcare environment of multidrug-resistant bacteria that are potential etiologies is still limited. Acknowledging that the antimicrobial potential may vary with geographical, pedological and anthropological determinants, investigating Cameroon variants appears very important. This combined knowledge could effectively guide the protocols used in controlling environmental bacterial populations and mitigating the rates of HAIs in many clinical settings.

The present work focused on the antimicrobial potential of the hydroethanolic extract obtained from dried leaves of *Cajanus cajan.* It was conducted on selected multidrug-resistant bacteria isolated from a healthcare environment where previous studies revealed high rates of antibiotic-resistant bacteria (Tchoukoua *et al*., 2018; Fotsing Kwetche *et al*., 2020; Menteng Tchuenté *et al*., 2023) and disinfectant-tolerant strains (chlorinated water (bleach), Surfanios®, iodine (Betadine®)) (Seuwo Koumwou and Fotsing Kwetche, 2020; Tsono and Fotsing Kwetche, 2020; Youté *et al*., 2024). It is done in the framework that encompasses the search for strategies and innovations that promote hygiene in healthcare facilities. The results will therefore guide the development of cheaper and available useful onsite policies in controlling the relevant microbial populations and mitigating the risk of HAIs.

2. material and methods

**2.1 Study design and ethical/administrative considerations**

This cross-sectional study was conducted from January 2nd to July 2nd, 2024. The plant material was collected in the Banekané neighbourhood (Ndé Division, West Cameroon), and the bacterial isolates were recovered from environmental specimens collected in the premises of the “Université des Montagnes” Teaching Hospital (UdMTH). The chemical procedures on the plant extract were performed in the Laboratory of Chemistry of the Université des Montagnes (UdM), and the microbiological screening in the laboratory of microbiology at the UdMTH

Prior to all investigations, necessary ethical and administrative requirements were fulfilled. Namely, they were the Ethical clearance Ref. N° 2024/024/UdM/PR/CEAQ delivered by the UdM ethical and quality Assurance Board, a research authorisation Ref. N° 2024/067/UdM/PR/DECANAT-ISSS/PHA and 2024/224/UdM/PR/DECANAT-ISSS/MED, provided by the academic authorities. These were followed by authorizations Ref N° 007/L/MINSANTE/SG/DRSPO/DSBgté and 2024/007/CUM/ADMN\_GENE, respectively provided by the Bangangté District Hospital and the UdMTH Heads.

**2.2 Setting up the useful bacterial pool**

**2.2.1 Sample collection, transport and storage**

The bacteria used in the present work were isolated from surfaces and from ambient air in the operating room, the shared external sanitation room, the childbirth room, the hospitalisation rooms, the biomedical analysis laboratory premises and the minor surgery room.

To collect surface bacteria, the modified wet swabbing procedure (Fotsing Kwetche *et al*., 2021, Youté *et al*., 2024) was used on the surfaces of beds, scialytic, hand-washing units, oxygenators, instrument tables, door handles, laboratory benches and baby weight scales. Ambient air bacteria were collected by passive sedimentation (Fotsing Kwetche *et al*., 2020; Menteng Tchuenté *et al*., 2023), on agar in Petri dishes with a contact time “agar-air” of 24 hrs. The culture media used contain Mannitol Salt, McConkey and Cetrimide agars. Specimen collection was done in the morning before the start of daytime activities, twice on separate days for each sample point. The collected specimens were immediately conveyed to the laboratory for microbial screening according to standard protocols.

**2.2.2 Bacterial screening of specimens**

Collected specimens were processed according to the usual principles (Denis *et al*., 2011) of standard analysis protocols, with *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 as reference bacterial strains used in quality control throughout the process of identification and susceptibility testing.

**2.2.2.1 Culture**

In the laboratory, Petri dishes with passively sedimented microbes were directly incubated aerobically for 24 hours at 37°C. For swabs, the cotton swab was aseptically broken off and immersed in a test tube containing 2 mL sterile physiological saline (0.9% NaCl), then vigorously shaken with a vortex agitator. Each resulting suspension was then inoculated onto Mannitol Salt, McConkey and Cetrimide agars using the streak-in-quadrant method. All inoculated media were thereafter incubated aerobically at 37°C for 24 hours.

**2.2.2.2 Bacterial identification**

After incubation, biochemical tests followed macroscopic and microscopic orientations in the identification of recovered isolates globally. Other identification variables depended on major bacterial types. Accordingly, the catalase, the DNase and the coagulase tests were used for Gram-positive cocci. For Gram-negative rods, the gallery of tests included the fermentation of carbohydrates (mannitol, glucose, and lactose), motility, citrate metabolism, urea degradation, indole production, tryptophanase production, H2S production, oxidase, and gas production. Identification of Gram-positive rods (GPR) was limited to microscopy.

**2.2.2.3 Antibiotic susceptibility tests**

After identification, each isolate was sub-cultured (37°C for 24h) on nutrient agar. With the 24 h-fresh population, the susceptibility tests were carried out by standard disk diffusion according to the “Comité de l'Antibiogramme de la Société Française de Microbiologie, EUCAST” (CASFM, 2023). Seventeen conventional antibacterial agents were used on the bacterial pool. Namely, they were Amoxicillin (20 or 25 µg), Amoxicillin/Clavulanic acid (20/10 µg), Aztreonam (30 µg), Cefixime (5 µg), Cefotaxime (5 µg), Cefoxitin (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Erythromycin (15 µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Penicillin G (10 U), Tetracycline (30 µg), Ticarcillin (75 µg), Trimethoprim/Sulfamethoxazole (1.75/23.25 µg). For the clinical categorisation of GPR isolates and the Penicillin G (10 U) testing, the 2013 recommendations of the CASFM (CASFM, 2013) were followed.

**2.3 Production of hydroethanolic extract from dried leaves of *Cajanus cajan***

**2.3.1 Harvesting and identification of plant material**

The *Cajanus cajan* leaves were collected in January 2024 from a farm in Banekané with the farm owner authorisation. Under the cover of identification certificate N° 063/IRAD/DG/CRA-MB/SS-BOT/SFlo-UR-PV/02/2024, these leaves were identified at the Cameroon National Herbarium, Yaoundé-Cameroon, as *Cajanus cajan* (L.) Millsp. (*Fabaceae*) In comparison with the original material reference N° 40324/HNC registered under N° 296.

**2.3.2 Hydroethanolic extraction (maceration)**

Koné *et al*. (2017) guidelines on factors influencing maceration [29] were observed in this preparation. First, the harvested leaves were carefully cleaned (cleared of debris, washed with clean water and drained). The cleaned leaves were dried in an oven (28°C and 61% humidity) reducing thereby, the total mass of the leaves from 8.6 kg to 2.3 kg.

After the dried leaves were completely ground, a fraction (500g) of crushed material was macerated for 48 h in 7 L of a hydroethanolic mixture (2.5 L distilled water + 4.5 L absolute ethanol) for two days. Subsequent to maceration, the mixture was filtered through a Whatman No. 1 paper. The resulting filtrate was dried at 45°C for 72 hours. The final product weighed 97.4 g for an extraction yield of 19.48%, and appeared as small green granules.

**2.4 Phytochemical screening**

This screening was carried out according to previous investigations (Fogue Totzo *et al*., 2023; Deumi Monthe *et al*., 2023). The groups of metabolites screened for were alkaloids, flavonoids, polyphenols, saponins, anthocyanins, catechic and gallic tannins, bound and free quinones, sterols and terpenes.

**2.5 Determining the minimal inhibitory, minimal bactericidal concentrations and categorising the extract activity**

This was carried out according to the 2-step scaled serial dilution principles (Fogue Totzo *et al*., 2023) with the following slight adjustments. The adjustments were:-1- The initial extract concentration used was 400 mg/mL; prepared in a 50% dimethyl sulfoxide solution. -2.1- The minimal inhibitory concentration (MIC) was determined on Müller-Hinton agar in glass test tubes maintained in a water bath (65°C) during the serial dilution procedure. Upon completion, the preparations were kept at room temperature where they solidified with a slope-2.2-over the medium slope, 15 µL of the bacterial inoculum, extemporary prepared at 0.5 McFarland, were streaked. These preparations in tubes were then incubated aerobically at 37°C for 24 hours. -2.3- Upon incubation completion, the MIC was identified as the lowest concentration of the extract for which no visible bacterial growth was recorded. -3- The minimal bactericidal concentration (MBC) was determined in liquid medium: two millilitres of sterile Müller-Hinton broth were dispensed in all dilutions where no visible growth was recorded during the MIC procedure, and all were reincubated at 37°C for 24 h. The MBC of the extract was eventually identified as the lowest concentration at which no turbidity was recorded in the overlaying broth.

Categorisation as bactericidal and bacteriostatic was finally done according to previous related protocols, guided by positive and negative control tests (Fogue Totzo *et al*., 2023).

**2.6 Data analysis**

The study's useful variables included bacterial groups, clinical categories (susceptible, susceptible at high dose, and resistant) of recovered isolates, the MICs of extract, the MBCs of extract and the MBC/MIC ratio. Their values were recorded and analysed with tools provided by the Microsoft Excel 2016 software. Bacterial groups were presented by sampling location. Clinical category results were presented as frequencies per bacterial type and antibacterial agents. It was used to present mean, standard deviation, minimum and maximum to present MICs, MBCs and MBC/MIC ratio per bacterial groups.

3. results

**3.1 Bacterial pool subjected**

The bacterial pool for the present study was made up of 64 isolates, predominantly *Staphylococcus* and Gram-positive rods. Less frequently recovered, Gram-negative rods were dominated by *Enterobacter* and *Pseudomonas* (9%, each). Additional related details are presented as displayed in Table 1. The overall picture reveals high bacterial diversity observed in five settings (laboratory, selected hospitalisation, minor surgery, hospitalisation and shared external sanitation room). Surfaces of hand-washing units and door handles were found to host the greatest bacteria diversity.

**Table 1. Distribution of bacterial types found according to premises**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Premises** | **Sample points** | ***Acinetobacter* spp.** | **GPR** | ***Enterobacter* spp.** | ***Klebsiella* spp.** | ***Pseudomonas* spp.** | ***Salmonella* spp.** | ***Serratia* spp.** | ***Staphylococcus* spp.** | ***Yersinia* spp.** |
| Operating room | Ambient air | - | 1 | - | - | - | - | - | - | - |
| Hand-washing unit | - | - | 1 | - | 1 | - | - | 1 | - |
| Bed | - | 1 | - | - | - | - | - | - | - |
| Oxygenator | - | 1 | - | - | - | - | - | 1 | - |
| Door handles | - | 1 | - | - | - | - | - | 2 | - |
| Scialytic | - | 1 | - | - | - | - | - | 1 | - |
| Instrument tables | - | - | 1 | - | - | - | - | 1 | - |
| Biomedical analysis laboratory | Ambient air | - | 1 | - | - | - | - | - | 1 | - |
| Hand-washing unit | - | 2 | - | 1 | 1 | - | - | 2 | 2 |
| Benches | - | 1 | - | - | 1 | - | - | 1 | - |
| Door handles | - | 1 | 1 | - | 1 | - | - | 1 | - |
| Childbirth room | Ambient air | - | 1 | - | - | - | - | - | 1 | - |
| Bed | - | 1 | - | - | - | - | - | - | - |
| Baby weigh scales | - | 1 | 1 | - | - | - | - | - | - |
| Instrument tables | - | 1 | - | - | - | - | - | - | - |
| Minor surgical room | Ambient air | - | - | - | - | - | - | - | 1 | - |
| Hand-washing unit | 1 | 1 | - | - | - | - | - | 1 | - |
| Bed | - | 1 | - | - | - | - | - | - | - |
| Door handles | - | 1 | 1 | - | - | - | - | 1 | - |
| Instrument table | - | 1 | - | - | - | - | - | 1 | - |
| Hospitalization room | Ambient air | - | - | - | - | - | - | - | 1 | - |
| Hand-washing unit | - | 1 | 1 | - | - | - | - | - | 1 |
| Bed | - | 1 | - | - | - | - | 1 | - | - |
| Door handles | - | 1 | - | 1 | 1 | - | - | - | - |
| Instrument table | - | 1 | - | - | - | - | - | 1 | - |
| Shared external sanitation room | Hand-washing unit | - | 1 | - | - | 1 | 1 | - | 2 | 1 |
| Total | | 1 | 23 | 6 | 2 | 6 | 1 | 1 | 20 | 4 |

GPR: Gram-Positive rods

This bacterial pool expressed high rates of resistance and multidrug resistance profiles. Table 2 indicates that the “susceptible at highdose” profile is rare in Gram-positive isolates, while resistance rates are invariably high for most antibiotics. Gentamicin was most effective, followed by Clindamycin on Gram-positive isolates.

**Table 2. Antibiotic susceptibility profile of isolates**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Antibiotics** | **GNR** | | | **GPR** | | | **GPC** | | |
| **S** | **SHP** | **R** | **S** | **SHP** | **R** | **S** | **SHP** | **R** |
| **Amoxicillin** | - | - | - | 9 | 0 | 91 | - | - | - |
| **Amox./a.clac.** | 8 | 17 | 75 | 0 | 0 | 100 | - | - | - |
| **Aztreonam** | 0 | 83 | 17 | - | - | - | - | - | - |
| **Cefixime** | 57 | 0 | 43 | - | - | - | - | - | - |
| **Cefotaxine** | 36 | 0 | 64 | - | - | - | - | - | - |
| **Ceftriaxone** | 21 | 0 | 79 | - | - | - | - | - | - |
| **Ciprofloxacin** | 67 | 5 | 29 | - | - | - | - | - | - |
| **Imipenem** | 0 | 0 | 100 | - | - | - | - | - | - |
| **Ticarcillin** | 14 | 0 | 86 | - | - | - | - | - | - |
| **Gentamicin** | 90 | 0 | 10 | 96 | 0 | 4 | 100 | 0 | 0 |
| **Levofloxacin** | 10 | 90 | 0 | 0 | 100 | 0 | 0 | 100 | 0 |
| **Tri./sulf.** | 57 | 0 | 43 | 0 | 0 | 100 | 65 | 0 | 35 |
| **Cefoxitine** | - | - | - | 0 | 0 | 100 | 25 | 0 | 75 |
| **Clindamycin** | - | - | - | 83 | 0 | 17 | 90 | 0 | 10 |
| **Erythromycin** | - | - | - | 19 | 0 | 81 | 70 | 0 | 30 |
| **Penicillin G** | - | - | - | 0 | 0 | 100 | 0 | 0 | 100 |
| **Tetracycline** | - | - | - | 39 | 0 | 61 | 60 | 0 | 40 |

-: Not tested; GNR: Gram-negative rods; GPR: Gram-positive rods; GPC: Gram-positive cocci; S: frequencies of susceptible isolates; SHP: frequencies of isolates susceptible at high posology; R: frequencies of resistant isolates; Amox./a.clac.:Amoxicillin/Clavulanic acid, Tri./sulf.:Trimethoprim/Sulfamethoxazole

**3.2 Groups of secondary metabolites in the extract**

The phytochemical screening was performed on the hydroethanolic extract obtained from the dried leaves of *Cajanus cajan* (L.) Millsp. revealed the presence of flavonoids, polyphenols, bound quinones, tannins and terpenes.

**3.3 The MIC, MBC and extract categories**

The minimal inhibitory and bactericidal concentrations (Table 3) of this hydroethanolic extract ranged from 1.465 through 93.75 mg/mL and from 6.25 through 175 mg/mL, respectively.

Further details (Table 3) indicate that this extract was most effective on *Staphylococcus* (MIC = 3.2663±1.6854 mg/mL; MBC = 12.0701±5.7882 mg/mL). Conversely, GPR were the least affected (MIC = 69.4185±17.4405 mg/mL; MBC = 116.8478±35.677 mg/mL) by the extract. Amongst the Gram-negative rods, *Pseudomonas* expressed the highest tolerance levels (MIC = 43.2292±3.0725 mg/mL; MBC = 73.9583±11.2962 mg/mL), followed by *Enterobacteriaceae* (MIC = 12.2342±3.6743 mg/mL; MBC = 22.1153±6.3016 mg/mL).

The results from Table 3 shown that the bacteriostatic potential was recorded on 70% of *Staphylococcus* and the bactericidal on 78.125% of the subjected isolates.

**Table 3. MICs and MBCs of the present extract of *Cajanus cajan* in relation to the present bacterial pool**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | | ***Acinetobacter* spp.** | **GPR** | ***Enterobacter* spp.** | ***Klebsiella* spp.** | ***Pseudomonas* spp.** | ***Salmonella* spp.** | ***Serratia* spp.** | ***Staphylococcus* spp.** | ***Yersinia* spp.** |
| **MIC (mg/mL)** | Average | 12.5 | 69.4185 | 11.4582 | 10.3515 | 43.2292 | 10.937 | 23.625 | 3.2663 | 11.8162 |
| Sta-Dev | - | 17.4405 | 1.2759 | 3.0384 | 3.0725 | - | - | 1.6854 | 3.0985 |
| Minimum | 12.5 | 43.5 | 9.375 | 8.203 | 37.5 | 10.937 | 23.625 | 1.465 | 8.203 |
| Maximum | 12.5 | 93.75 | 12.5 | 12.5 | 46.875 | 10.937 | 23.625 | 8.203 | 15.625 |
| **MBC (mg/mL)** | Average | 25 | 116.8478 | 21.875 | 17.9685 | 73.9583 | 21.875 | 31.5 | 12.0701 | 22.263 |
| Sta-Dev | - | 35.677 | 4.8412 | 9.944 | 11.2962 | - | - | 5.7882 | 8.5032 |
| Minimum | 25 | 62.5 | 12.5 | 10.937 | 62.5 | 21.875 | 31.5 | 6.25 | 10.927 |
| Maximum | 25 | 175 | 25 | 25 | 87.5 | 21.875 | 31.5 | 32.812 | 31.25 |
| **MBC/MIC** | Average | 2 | 1.73 | 1.95 | 1.65 | 1.72 | 2 | 1.3 | 4.15 | 1.83 |
| Sta-Dev | - | 0.51 | 0.54 | 0.49 | 0.32 | - | - | 1.73 | 0.35 |
| Minimum | 2 | 1 | 1 | 1.3 | 1.3 | 2 | 1.3 | 2 | 1.3 |
| Maximum | 2 | 2.7 | 2.7 | 2 | 2 | 2 | 1.3 | 8 | 2 |

GPR: Gram-positive rods; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; Sta-Dev: Standard deviation

4. discussion

In the framework that aims at valorising plant derivatives as alternatives to conventional antibacterial agents, the present investigation assessed the antibacterial potential of a hydroethanolic extract from the dried leaves of *Cajanus cajan* on a pool of antibiotic-resistant bacteria recovered from a healthcare facility environment. The subjected bacterial pool consisted primarily of *Staphylococcus* and *Bacillus*, though the isolation spectrum extended to *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. Higher bacterial diversity was recorded in the biomedical laboratory, the minor surgery room, the hospitalisation wards, the shared external sanitation rooms, on the surfaces of hand-washing units and door handles. These settings are commonly used by healthcare personnel, patients and visitors and deserve appropriate hygiene practices for optimal safety. The bacterial population subjected to susceptibility tests expressed high rates of multidrug resistance. These diversity and susceptibility profiles were in line with previous findings in the same healthcare facility (Tchoukoua *et al*., 2018; Fotsing Kwetche *et al*., 2020; Menteng Tchuenté *et al*., 2023; Youté *et al*; 2024) and in the others (Tchapdie Ngassam *et al*., 2017).

The predominance of Gram-positive bacteria could, at first glance, reflect cell stability conferred by the chemical composition of the cell envelope, but also their ability to withstand harsher environmental stresses with a spore in *Bacillus*. These characteristics are shared by the major categories recovered and appear consistent with the reduced isolation rates of Gram-negative bacteria. As ubiquitous facultative aerobes, a higher likelihood to contaminate and cause opportunistic infections to more vulnerable exposed humans could reasonably be anticipated (Albert *et al*., 2000; Fotsing Kwetche *et al*., 2020; Souza *et al*., 2021;Menteng Tchuenté *et al*., 2023; Youté *et al*, 2024). Consistent with previous authors' conclusions (Fotsing Kwetche *et al*., 2020; Menteng Tchuenté *et al*., 2023; Youté *et al*, 2024), these bacterial types could effectively be regarded as reliable indices of what healthcare environment hygiene initiatives could build, particularly in contexts where resources for in-depth analyses are limited (Fotsing Kwetche *et al*., 2020; Menteng Tchuenté *et al*., 2023; Youté *et al*, 2024).

The great diversity observed at various sampling points could be justified by human frequentation (staff, patients, visitors) and the diversity of frequent human activities (Fotsing Kwetche *et al*., 2020; Menteng Tchuenté *et al*., 2023). In fact, these premises and materials (hand-washing units and door handles) are frequently used in healthcare, implying that designing policies for optimal hygiene should be rethought in this context.

The high resistance rates observed in the present study could predict caretaking failure in the case of infections acquired in the UdMTH. These resistance rates could initially be attributed to probabilistic use of antimicrobial agents in community and/or hospital, due to economic constraints and resource limitations endured by local populations (Tchoukoua *et al*., 2018; Fotsing Kwetche *et al*., 2020; Menteng Tchuenté *et al*., 2023). These constraints are part of the reasons for the frequent uncontrolled use of first-line drugs with a broad spectrum of action, such as beta-lactams, in connection with their availability, affordability and administration routes. Acknowledging that the routine probabilistic and empiric use of these drugs is core justification for resistant growth, reinforcing individual knowledge and overall regulation appears as a paramount necessity in preventing microbial resistance exacerbation. In this regard, the GNR profile to imipenem emerges as a puzzle. Not usually recommended, the expectation would be high effectiveness. The related resistance rate recorded resists clear explanation, but could be in connection with frequent administration of other antimicrobials that select resistance from complex mobile genetic determinants in cross or co-resistance within the polymicrobial population in hospital environments, and consistent with the necessity to reinforce policies for better contextual hygiene (Souza *et al*., 2021). This gap should be filled to anticipate the effectiveness of therapies with antibiotics sustainably. In this vein, investigations through new alternatives like the current one on *Cajanus cajan* extract should relentlessly be encouraged (Okigbo and Omodamiro, 2007; Kong *et al*., 2010; Nwachukwu and Uzoeto, 2010; Oyewole *et al*., 2010; Pal *et al*., 2011; Pratima and Mathad, 2011; Ahomadegbe *et al*., 2018; Gargi *et al*., 2022; Yilwa *et al*., 2023), primarily for environmental hygiene.

The phytochemical screening of the hydroethanolic extract of dried leaves of *Cajanus cajan* (L.) Millsp. revealed the presence of flavonoids, polyphenols, bound quinones, tannins and terpenes, and the absence of alkaloids, saponins, anthocyanins and sterols. Several previous studies reported the presence of alkaloids, flavonoids, terpenoids, steroids, phytosteroids, saponins, tannins, phenol, anthraquinones, quinones, xanthoproteins, phlobatannin, coumarins, stilbenoids from these plants’ leaves (Okigbo and Omodamiro, 2007; Kong *et al*., 2010; Nwachukwu and Uzoeto, 2010; Oyewole *et al*., 2010; Pal *et al*., 2011; Pratima and Mathad, 2011; Ahomadegbe *et al*. 2018; Gargi *et al*., 2022; Yilwa *et al*., 2023). The absence of some phytochemicals in the present research may be related to the extraction protocol (Moroh *et al*., 2008; Bagre *et al*., 2011; Nwachukwu and Uzoeto, 2010; Pratima and Mathad, 2011; Fogue Totzo *et al*., 2023; Yilwa *et al*., 2023). It may also be linked with geographic, pedologic and anthropologic determinants that could vary with the raw material collection sites (Okigbo and Omodamiro, 2007; Garcia-Salas *et al*., 2010; Kong *et al*., 2010; Nwachukwu and Uzoeto, 2010; Oyewole *et al*., 2010; Pal *et al*., 2011; Pratima and Mathad, 2011; Ahomadegbe *et al*., 2018; Gargi *et al*., 2022; Yilwa *et al*., 2023).

The minimal inhibitory and bactericidal concentrations of the hydroethanolic extract of *Cajanus cajan* (L.) Millsp. leaves ranged from 1.465 through 93.75 mg/mL and from 6.25 through 175 mg/mL, respectively. This extract was more effective (inhibitory and bactericidal effectiveness) on *Staphylococcus* (MIC = 3.2663±1.6854 mg/mL; MBC = 12.0701±5.7882 mg/mL). This effectiveness was found to be drastically reduced on Gram-positive rods (MIC = 69.4185±17.4405 mg/mL; MBC = 116.8478±35.677 mg/mL). Amongst the Gram-negative rod isolates, the highest MIC and MBC values were recorded with *Pseudomonas* (MIC = 43.2292±3.0725 mg/mL; MBC = 73.9583±11.2962 mg/mL). Combined, these findings deserve closer attention to understand what bacterial features of susceptibility or resistance. At first glance, however, it seems not to be solely related to the chemical composition of the bacterial cell envelope as observed above. More insight could anticipate the role of spores in GPR, which likely provide additional protection. These inhibitory potentials are better expressed than those reported in a previous study (Pratima and Mathad, 2011) when chloroform or petroleum ether was used for extraction, but not as well as reported with the methanolic, ethanolic, and aqueous extracts (MICs globally recorded at 6.25 mg/mL). However, these inhibitory and bactericidal levels were better than those obtained with methanolic, ethanolic, acetonic, hot water and cold water extracts as reported by Nwachukwu and Uzoeto(2010).

The antibacterial activity in the present study could be justified (at least partially) by the chemical composition in terms of secondary metabolites groups, according to several previous studies as reported in literature (Elizondo *et al*., 2010; Cushnie *et al*., 2014; Bakrim *et al*., 2022; Dahlem Junior *et al*., 2022; Gargi *et al*., 2022; Hamdi Abdulkareem *et al*., 2022; Shamsudin *et al*., 2022; Wiart *et al*., 2023; Sharma *et al*., 2024; Li *et al*., 2024; Deng *et al*., 2024). Other factors related to unidentified metabolites and stochastic chemical interactions likely impact the findings, as they basically occur naturally to ensure plant survival and fitness.

The variation in MICs and MBCs observed highlights that microbial tolerance to antimicrobials also depends on the individual microbial characteristics. Accordingly, in addition to how the extracts act on microorganisms, it would be important to understand what strategies should be employed to overcome the mechanisms that target microbes use to withstand the action of the extract they are exposed. Based on the antibacterial potentials of *Cajanus cajan* (L.) Millsp observed in the present survey, the diversity of bacterial isolates exposed to its hydroethanolic extract and inhibitory values recorded should motivate an initiative towards enhancing the prospect through clinical categorisation for this plant extract as done with conventional antibiotics. The bacterial group-dependent values (*Staphylococcus*: MIC = 3.2663±1.6854 mg/mL; CMB = 12.0701±5.7882 mg/mL; *Acinetobacter*: MIC = 12.5 mg/mL; CMB = 25 mg/ml; *Enterobacteriaceae*: MIC = 12.2342±3.6743 mg/mL; BMC = 22.1153±6.3016 mg/mL; *Pseudomonas*: MIC = 43.2292±3.0725 mg/mL; BMC = 73.9583±11.2962 mg/mL; GPR: MIC = 69.4185±17.4405 mg/mL; MBC = 116.8478±35.677 mg/mL) are clear indications that this categorization can be achieved. Once done, it would help to build ranges of extract concentrations that could be used for hospital surface hygiene or other protocols that require bacterial selection.

The bactericidal potential of the extract, which was found to be 78.125%, is consistent with the conclusions on the selective role it could play. All the above pieces of information from *Cajanus cajan* robustly suggest it as a proper candidate for traditional drugs or disinfectants alternative in several contexts, as previously reported(Youté *et al*., 2024; Seuwo Koumwou and Fotsing Kwetche, 2020; Tsono and Fotsing Kwetche, 2020). In fact, it was found that selection of resistance with plant extract would be less common (if ever) (Bouyahya *et al*., 2022) and that *Cajanus cajan* extract was significantly safer for the users (Ahomadegbe *et al*., 2018). Therefore, it could be used in controlling bacterial populations not only in healthcare facilities, but also in animal farms and in households where selective agents are commonly used to prevent infections.

5. Conclusion

The present study on the antibacterial potential of the hydroethanolic extract from the dry leaves of *Cajanus cajan* (L.) Millsp on multidrug-resistant bacteria recovered from the healthcare facility environment revealed the highest effectiveness on *Staphylococcus* and the lowest on Gram-positive rods. With the Gram-negative rod populations, extract effectiveness was drastically reduced on *Pseudomonas,* compared to members of the *Enterobacteriaceae* family. Acknowledging that Gram-positive cocci and Gram-positive rods are common colonisers of healthcare environments and indices that can serve contextual hygiene, additional investigations could make this extract a suitable candidate to be used in controlling bacterial populations in healthcare settings.

Data availability

Data associated with this work were not deposited into a publicly available repository. All the data of this work are present in this paper.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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