Original Research Article

Antimicrobial and Phytochemical Studies of Hydroethanolic Extracts of *Plumbago zeylanica* (L.), a Medicinal Plant Used against Microbial Infections and Intestinal Disorders

.

ABSTRACT

|  |
| --- |
| Antibiotic resistance remains a real public health problem. The search for new molecules to effectively combat this problem is becoming a necessity. The objective of this study was to evaluate the *in vitro* antimicrobial effect of hydroethanolic extracts of leaves and roots of *Plumbago zeylanica* and to highlight the phytochemical compounds present in these extracts. Reference strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853) and clinical isolates (*Escherichia coli* ESBL, *Shigella spp*, *Klebsiella spp*, *Acinetobacter spp*, *Pseudomonas aeruginosa*, *Enterococcus spp*, *Staphylococcus aureus* MRSA and *Candida albicans*) were used. The agar well diffusion and broth microdilution methods were used to evaluate the antimicrobial effect. The susceptibility of the strains varied, depending on the extract, with a best inhibitory effect of the hydroethanolic extract of *P. zeylanica* roots on *S. aureus* ATCC 29213, *S. aureus* MRSA, *Shigella spp* and *C. albicans*. The MICs obtained was between 0.63 and 5 mg/ml. The most effective antimicrobial potential was obtained with the roots extract and staphylococcal strains were the most sensitive to the tested extracts. The tested extracts contain compounds such as flavonoids, tannins, phenolic compounds, triterpenes and sterols, saponosides and cardenolipid glycosides. These results support the use of *P. zeylanica* for the treatment of microbial diseases and contribute to the search for new bioactive molecules. |

Keywords:Antimicrobial resistance, *Plumbago zeylanica*, leaves and roots extracts, antimicrobial potential, phytochemicals.

1. INTRODUCTION

For several decades, antibiotic therapy has been the incontestable means of combating disastrous situations linked to infectious diseases and restoring health (Okou et al., 2018). The use of antibiotic molecules in human and animal health has thus been marked by relief for populations threatened with disability and sometimes death from epidemics and microbial diseases considered incurable (Vasseur, 2014). At the same time, the prescription of medicinal plants by local herbalists to combat diseases was diverted in favor of synthetic molecules promoted by conventional medicine. However, the use of these new synthetic molecules very quickly resulted in clinical failures (Kagnou et al., 2020; Ouro-Djeri et al., 2022). Their effectiveness is continually eroded following the spread of antibiotic resistance genes with the immediate effect of increasing the number of deaths and the socio-economic consequences associated with infectious diseases (Ouedraogo et al., 2017). This phenomenon, essentially accentuated by the misuse of antimicrobial molecules in human health and in the environment, favors the selection of resistant mutants and concerns all microbial strains involved in the occurrence of infectious pathologies (Gan et al., 2024). Multidrug-resistant strains have been identified in communities and in healthcare structures (Dos Santos et al., 2024). A large number of *S. aureus* responsible for post-surgical suppurations and abscesses have been shown to be resistant to cephamycins and vancomycin. Carbapenem-resistant Enterobacteriaceae have been recorded in many hospitals around the world (Epelboin et al., 2015; Sohrabi et al., 2024).

To overcome this situation, which comes on top of the relatively high cost of conventional molecules, many hopes remain placed in the secret of medicinal plants (Kagnou et al., 2020). They present, through their diversity, the greatest resource of bioactive natural substances that can act alone or in synergy on molecular targets, inhibit, interfere in cellular metabolism or sequester quorum sensing auto-inducers to thus counteract resistance mechanisms in microorganisms (Bouyahya et al., 2017; Mignanwandé et al., 2020).

*Plumbago zeylanica* L. (Plumbaginaceae) is a leafy subshrub native to South Asia. The leaves and roots of this plant are used by traditional medicine in decoction, dyeing or powder in Africa and Asia to combat skin diseases, microbial conditions such as leprosy, scabies, ringworm, dermatitis, abscesses, boils, itchy skin, diarrhoea, sores, leg ulcers and post-surgical suppurations (Singh et al., 2017; Shukla et al., 2021). Despite these uses in traditional medicine, little scientific work has confirmed the pharmacological effects of this plant. It is in this mindset of valorization of plant species of the African flora that the present study was undertaken with a view to evaluating the *in vitro* antimicrobial potential of hydroethanolic extracts of the roots and leaves of *P. zeylanica* on reference strains and clinical isolates involved in the occurrence of infectious pathologies in humans.

2. material and methods

**2.1 Plant material**

The plant material used consisted of leaves and roots of *P. zeylanica*. These organs, collected in Avetonou at Agou area in May 2023, were botanically identified at the Laboratory of Botanic and Plant Ecology, Faculty of Sciences, University of Lomé. These plant organs were carefully washed, dried at air condition room for two weeks, then crushed into powder (Hoekou et al., 2015).

**2.2 Microbial strains**

The reference strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853) were provided by the National Institute of Hygiene of Lomé. The clinical germs were isolated from pus in the Bacteriology Laboratory of Saint John of God Hospitalin Afagnan. The isolated microorganisms are: *E. coli* extended-spectrum beta-lactamase (ESBL), *Shigella spp*, *Klebsiella spp*, *Acinetobacter spp*, *P. aeruginosa, Enterococcus spp*, Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans*.

**2.3 Extractions**

The extraction was performed by maceration of 100 g powder from each organ in 1000 ml of hydroethanolic solution (30/70). This mixture obtained was stirred manually for 5 minutes and then left under continuous stirring for 72 hours at room temperature. The macerate obtained was successively filtered twice through hydrophilic cotton then once through Whatman N°1 filter paper and evaporated under vacuum at 40°C and at reduced pressure between 999-1000 in a rotavapor. The extracts thus collected were stored at +4°C in sterile and airtight boxes until their use (Tidiane et al., 2021).

**2.4 Phytochemical screening**

The major phytochemical groups were revealed in this study by chemical tests carried out on the two hydroethanolic extracts of *P. zeylanica* according to Harbone, (1991) and EL-Haoud et al. (2018). These methods were essentially based on the principles of solubility, coloring and precipitation making it possible to highlight the presence of the main secondary metabolites.

**2.4.1 Test for phenolic compounds**

The reaction with ferric chloride (FeCl3) made it possible to characterize the polyphenols. To 2 ml of each hydroethanolic extract, add a drop of 2% alcoholic solution of ferric chloride. The appearance of a more or less dark blue-blackish or green color confirmed a sign of the presence of polyphenols.

**2.4.2 Test for alkaloids**

The alkaloids were characterized using Burchard reagents (iodo-iodide reagent) and Dragendorff reagents (potassium iodo-bismuthate reagent). 6 ml of each solution were evaporated to dryness. The residue was dissolved in 6 ml of 60° alcohol. The addition of 2 drops of Dragendorff's reagent to the alcohol solution caused a precipitate or an orange color. Adding 2 drops of Burchard's reagent to the alcohol solution caused a reddish-brown precipitate and indicated a positive reaction.

**2.4.3 Test for flavonoids**

Flavonoids were investigated by the cyanidin reaction. 2 ml of each extract were evaporated and the residue was taken up in 5 ml of hydrochloric alcohol diluted twice. By adding 2 to 3 magnesium shavings, heat was released followed by a pink-orange or purplish color. The addition of 3 drops of isoamyl alcohol intensified this coloring which confirmed the presence of flavonoids.

**2.4.4 Test for reducing compounds**

Their detection consists of introducing 2 ml of the extract into a test tube and 2 ml of Fehling liqueur is added. Then, the whole is brought to a boiling water bath for 8 min. Obtaining a brick red precipitate indicates the presence of reducing compounds.

**2.4.5 Test for cardenolipid glycosides**

2 ml of chloroform is added to 1 ml of the extract, the appearance of a reddish-brown color after the addition of H2SO4 indicates the presence of cardenolipid glycosides.

**2.4.6 Test for tannins**

The presence of tannins is demonstrated by adding to 1 ml of each extract, 1 ml of water and 1 to 2 drops of Fecl3 solution diluted to 1%. The appearance of a dark green or blue-green color indicates the presence of tannins. The appearance of a dark green color indicates the presence of catechic tannins. The appearance of a blue-green color indicates the presence of gallic tannins.

**2.4.7** **Test for saponosides**

To test for saponosides, 10 ml of the hydroethanolic extract were poured into a test tube. The tube was shaken for 15 secondes then left to rest for 15 min. A persistent foam height greater than 1 cm indicated the presence of saponosides.

**2.4.8 Test for sterols and triterpenes**

Sterols and polyterpenes were sought by the Liebermann reaction. 5 ml of each extract were evaporated on a sand bath. The residue is dissolved hot in 1 ml of acetic anhydride; 0.5 ml of concentrated sulfuric acid was added to the triturate. The appearance, at interphase, of a purple or violet ring, turning blue then green, indicated a positive reaction.

**2.4.9 Test for quinones**

A few drops of 1/10 NaOH were added to a tube containing 2 ml of extract to be analyzed. The presence of quinones is confirmed when the aqueous phase turns yellow, red or purple.

**2.5 Inoculum preparation**

The inoculum of the strains identified for the study was prepared from 24 hours young colonies taken from Mueller Hinton or Sabouraud chloramphenicol agar and suspended into 10 ml of physiological water then adjusted to 0.5 Mac Farland. This inoculum was used to inoculate the agar plates by swabbing (Lagnika et al., 2016).

**2.6 Sensitivity tests of strains to plant extracts**

The agar well-diffusion method was used to investigate the sensitivity of the microbial strains to the extracts. Mueller Hinton and Sabouraud Chloramphenicol agars were used for bacteria and *C. albicans*, respectively (Hoekou et al., 2015). A solution of 20 mg/ml of each extract was prepared by dissolving the dry extract in dimethylsulfoxide 1% (DMSO). Wells of 6 mm in diameter were made in each petri dishes previously inoculated. 50 μl of each extract solution at 20 mg/ml were dispensed into these wells. Each plate was made in duplicate. The plates were then left for 30 minutes to 1 hour at room temperature then incubated at 37°C for 18 - 24 hours. Gentamicin (10 µg) and nystatin (100 µg) were used as positive controls for bacteria and yeast, respectively (Chaouche et al., 2016). DMSO 1% in sterilized distilled water served as a negative control. The sensitivity of the germs to the extracts was evaluated by measuring inhibition zone diameters around the wells.

**2.7 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

The minimum inhibitory concentration (MIC) was determined by microdilution method in 96 well-plates with Mueller Hinton broth for bacteria and Sabouraud broth for *C. albicans*. The microbial suspensions were diluted 10-2 with broth and dispensed into 96 well microplates containing a range of concentrations of each extract from 20 to 0.156 mg/ml. The plates were incubated at 37°C for 24 hours for bacteria and 48 hours for yeast. The MIC was determined as the lowest concentration of extract demonstrating no visible growth on the broth (NCCLS, 2003; Chibuzor et al., 2024).

The minimum bactericidal concentration of the extracts was determined after reading the MIC. The starting inoculum was diluted from 10-1 to 10-4. These different solutions were inoculated onto Muller Hinton agar in 5 cm strips using a 2 µl loop, then incubated at 37°C for 24 hours. At the same time, the contents of all wells in which there was no visible growth were inoculated on Mueller Hinton agar starting from the MIC towards the highest concentrations, then incubated at 37°C for 24 hours. The MBC was determined after counting the colonies as being the lowest concentration of the extract that allowed less than 0.01% of the bacteria in the starting inoculum to survive (Sina et al., 2021).

**2.8 Determination of the antimicrobial power of plant extracts (MBC/MIC)**

The MBC/MIC report made it possible to highlight the modalities of action of the plant extracts studied. A substance is said to be bactericidal when the MBC/MIC ratio ≤ 4; bacteriostatic when this ratio is greater than 4; and ineffective or tolerant on the strain studied when this ratio is greater than 32 (Sina et al., 2021).

**2.9 Data analysis**

The data recorded during the antimicrobial tests were processed using Excel 2016. The results are expressed as mean ± SEM (Standard Error of the Mean).

3. results

**3.1 Extraction and phytochemical screening**

The extraction yields were 7.25% for the hydroethanolic extract of the leaves and 6.0% for that of the roots of *P. zeylanica*.

The preliminary phytochemical study revealed the presence of flavonoids, gallic and catechic tannins, phenolic compounds, sterols and triterpenes, cardenolipid glycosides and saponosides in the two hydroethanolic extracts of *P. zeylanica* studied. Reducing compounds and alkaloids were found only in the hydroethanolic extract of *P. zeylanica* leaves, whereas quinones were absent in the two extracts (Table 1).

**Table 1: Results of phytochemical screening**

|  |  |  |
| --- | --- | --- |
| **Secondary metabolites** | **Hy-Ext Ra** | **Hy-Ext Fe** |
| Flavonoids | **+** | **+** |
| Phenols compounds | **+** | **+** |
| Cardenolipid glycosides | **+** | **+** |
| Catechical tannins | **+** | **+** |
| Gallic tannins | **+** | **+** |
| Alkaloids | **-** | **+** |
| Saponosides | **+** | **+** |
| Sterols and Triterpenoids | **+** | **+** |
| Quinones | **-** | **-** |
| Reducing compounds | **-** | **+** |

+ : Presence; - : Absence, Hy-Ext Ra = Hydroethanolic Roots extract,

Hy-Ext Fe = Hydroethanolic Leaves extract.

**3.2 Sensitivity of strains to tested extracts**

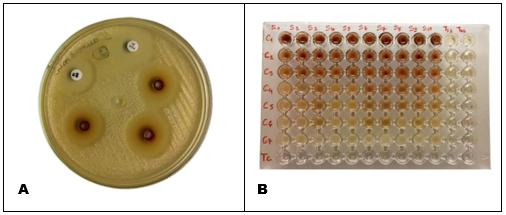
The different inhibition zone diameters obtained during the tests appear in Table 2 and Figure 1A. The hydroethanolic extracts of the leaves and roots of *P. zeylanica* had variable effects both on the reference strains and on the clinical isolates tested. However, the root extract was more active in inhibiting the growth of all reference strains and some clinical isolates studied with inhibition zone diameters that varied from 15 to 26 mm. The highest inhibition zone was obtained on *S. aureus* ATCC 29213 (26 mm) at the concentration of 20 mg/ml. The root extract inhibited the growth of the Gram-negative bacilli isolates (*Shigella spp*, *E coli* ESBL, *P. aeruginosa*) with the exception of *Klesbsiella spp* and *Acinetobacter spp*. This extract has also been shown to be effective against *S. aureus* MRSA. Besides, the hydroethanolic extract of the leaves was only found to be active at the same concentration on *S. aureus* ATCC 29213 (19 mm) among the reference strains and on *S. aureus* MRSA (16 mm). On the other hand, it was inactive on *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. coli* ESBL, *Shigella spp*, *Klebsiella spp*, *Acinetobacter* *spp* and *P. aeruginosa*. Both extracts had no effect on *Enterococcus spp*. *C. albicans* used for this work was inhibited by the hydroethanolic extract of *P. zeylanica* roots while the extract of the leaves was inactive on the same isolate. The reference molecules, gentamicin (10 μg/ml) for the bacterial strains and nystatin (100 μg/ml) for *C. albicans*, also inhibited the growth of the strains used in this study with inhibition zone diameters varied from 9 to 20 mm (Table 2).

**Table 2**: **Sensitivity of the strains studied to plant extracts**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Microbial strains** | **Inhibition Zone Diameters (in mm)** | | | |
| **Hy-Ext Ra** | **Hy-Ext Fe** | **GEN 10 µg** | **NS 100 µg** |
| *S. aureus* ATCC 29213 | 26 ± 0.20 | 19 ± 0.00 | 18 ± 0.30 | NT |
| *P. aeruginosa* ATCC 27853 | 16 ± 0.00 | 0 ± 0.00 | 16 ± 0.10 | NT |
| *E. coli* ATCC 25922 | 15 ± 0.10 | 0 ± 0.00 | 18 ± 0.00 | NT |
| *Shigella spp* | 22 ± 0.00 | 0 ± 0.00 | 20 ± 0.30 | NT |
| *Klebsiella spp* | 0 ± 0.00 | 0 ± 0.00 | 14 ± 1.00 | NT |
| *Acinetobacter spp* | 0 ± 0.00 | 0 ± 0.00 | 13 ± 0.00 | NT |
| *E. coli BLSE* | 15 ± 0.03 | 0 ± 0.00 | 17 ± 0.10 | NT |
| *S. aureus* SARM | 22 ± 0.00 | 16 ± 0.10 | 19 ± 0.40 | NT |
| *Enterococcus spp* | 0 ± 0.00 | 0 ± 0.00 | 9 ± 0.20 | NT |
| *P. aeruginosa* | 15 ± 0.10 | 0 ± 0.00 | 16 ± 0.00 | NT |
| *Candida albicans* | 19 ± 0.10 | 0 ± 0.00 | NT | 19 ± 0.50 |

Hy-Ext Ra = Hydroethanolic Roots extract, Hy-Ext Fe = Hydroethanolic Leaves extract,

GEN = Gentamicin, NS = Nystatin, NT = Not tested.

****

**Figure 1: Results of the sensitivity test of hydroethanolic extract of *P. zeylanica* Root on *S. aureus* ATCC 29213 at the left (A), and the determination of the MICs at the right side (B).**

**3.3 Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of the active extracts**

Antimicrobial parameters were presented on Table 3. The MIC of the hydroethanolic extract of *P. zeylanica* roots varied from 0.62 to 1.25 mg/ml on the reference strains. They were 0.62 to 5 mg/ml against the Gram-negative bacilli (*E. coli*, *Shigella spp*, *Acinetobacter spp*) and the Gram-positive cocci (*S. aureus*). It was 1.25 mg/ml still with the hydroethanolic extract of *P. zeylanica* roots on *C. albicans*. On the other hand, the MIC of the hydroethanolic extract of *P. zeylanica* leaves were 5 mg/ml on *S. aureus* ATCC 29213 and *S. aureus* MRSA. The roots extract of *P. zeylanica* was more active on the growth of the strains used compared to the antimicrobial effect revealed by the hydroethanolic extract of the leaves. The lowest MIC is obtained on *S. aureus* ATCC 29213 and *E. coli* ESBL with the hydroethanolic extract of *P. zeylanica* roots. The MBC of the hydroethanolic extract of *P. zeylanica* roots on bacteria ranged from 1.25 to 20 mg/ml. On *C. albicans*, the MBC was 5 mg/ml. The MBC of the hydroethanolic extract of *P. zeylanica* leaves was 20 mg/ml on the two strains of *S. aureus* studied. The roots extract showed bactericidal effect on the tested bacteria except *P. aeruginosa*. The same extract was fungicidal on *C. albicans.* The leaves extract was bactericidal on the two strains of *S. aureus.*

**Table 3:** **Antimicrobial parameters: MIC, MBC and antimicrobial potency**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Microbial strains** | **Types of extracts** | **MIC**  **(mg/ml)** | **MBC**  **(mg/ml)** | **MBC/MIC** | **Activity** |
| *S. aureus ATCC 29213* | Hy-Ext Ra | 0.63 | 2.50 | 3.96 | Bactericidal |
| *P. aeruginosa ATCC27853* | Hy-Ext Ra | 1.25 | 5.00 | 4.00 | Bactericidal |
| *E. coli ATCC25922* | Hy-Ext Ra | 0.63 | 1.25 | 1.90 | Bactericidal |
| *Shigella spp* | Hy-Ext Ra | 5.00 | 10.00 | 2.00 | Bactericidal |
| *S. aureus SARM* | Hy-Ext Ra | 5.00 | 10.00 | 2.00 | Bactericidal |
| *E. coli* BLSE | Hy-Ext Ra | 0.63 | 2.50 | 3.96 | Bactericidal |
| *P. aeruginosa* | Hy-Ext Ra | 1.25 | 10.00 | 8.00 | Bacteriostatic |
| *S. aureus* SARM | Hy-Ext Fe | 5.00 | 20.00 | 4.00 | Bactericidal |
| *S. aureus ATCC*29213 | Hy-eth Fe | 5.00 | 20.00 | 4.00 | Bactericidal |
| *C. albicans* | Hy-Ext Ra | 1.25 | 5.00 | 4.00 | Fungicidal |

Hy-Ext Ra = Hydroethanolic roots extract; Hy-Ext Fe = Hydroethanolic leaves extract;

MIC = Minimal inhibitory concentration; MBC = Minimal bactericidal concentration.

**4. DISCUSSION**

Plants constitute a potential source of natural molecules essential for humans to prevent diseases, restore their health, feed and protect or even beneficial for other forms of life. The understanding of the pharmacological properties of phytoconstituents and the identification of new molecules of medical interest remain obvious for communities threatened with disability and sometimes death by these pests which are microorganisms. The leaves and roots of *P. zeylanica* are used in dyeing, poultices and decoctions by traditional medicine in the plateau and maritime regions of Togo to combat microbial diseases in humans and damage to the skin and mucous membranes in domestic animals and livestock. This traditional use guided the choice of hydroethanolic extraction adopted in this study using ethanol and water as solvents (Agban et al., 2020). Furthermore, this mixture of polar solvents allows the extraction of a large number of molecules depending on its affinity towards the secondary metabolites present within the plant (Tidiane et al., 2021). A fairly large yield without variability was recorded in this study during the hydroethanolic extraction carried out on the leaves and roots of *P. zeylanica*.

During the present work, carried out on the hydroethanolic extracts of the leaves and roots of *P. zeylanica*, the search for the major phytochemical groups revealed the presence of flavonoids, tannins, phenolic compounds, terpenoids and sterols in the two plant extracts while the alkaloids were found only in the hydroethanolic extract of the leaves of *P. zeylanica*. Shukla et al. (2021) reported the presence of alkaloids in the roots of *P. zeylanica*. These authors indicated that in addition to alkaloids, quinones were strongly represented within the plant. There was a difference between our results and those of these authors. This can be explained by a difference in several parameters, whether geographical, physicochemical or biological, such as: the difference in the harvest site including the environment of the plant, light, precipitation, topography, season, type of soil, harvest period, the genetic heritage of the plant or the extraction procedure used (Hoekou et al., 2015; EL-Haoud et al., 2018).

Susceptibility tests carried out with hydroethanolic extracts of the leaves and roots of *P. zeylanica* showed that the sensitivity of reference strains and clinical isolates varied from one germ to another depending on the extract. The hydroethanolic extract of *P. zeylanicaun* roots revealed a significant inhibitory effect against the growth of the majority of reference strains and clinical isolates. On the other hand, the hydroethanolic extract of leaves of *P. zeylanica* was only found to be active against *S. aureus* ATCC 29213 and *S. aureus* MRSA. The results were similar to those reported by Singh et al. (2017). These authors had meant that the alcoholic extracts of the roots of *P. zeylanica* had a greater inhibitory action compared to that revealed by the alcoholic extracts of the leaves. The variability of the antimicrobial effect between the two extracts of *P. zeylanica* on the reference strains and the clinical isolates could be explained by the variation in the concentration of active principle passing from one organ to another within the same plant. Indeed, plants contain, in varying proportions, secondary metabolites such as phenolic compounds, alkaloids or sterols (Kagnou et al., 2020; Mignanwandé et al., 2020). These molecules with multiple properties can cross cell membranes and interact with structural constituents, interfere with metabolism in microorganisms or act by inhibiting gene products to thus sabotage their growth (Bouyahya et al., 2017). Zhu et al. (2022) reported that the two alcoholic extracts of roots and leaves of *P. zeylanica* were active against bacteria. However, these authors used the plant extracts at a higher dose (100 mg/ml) than that adopted for this study (20 mg/ml). This could explain the divergence of our results regarding the sensitivity of clinical isolates of *Klebsiella spp*, *Acinetobacter spp* and *Enterococcus spp* against the hydroethanolic extract of roots of *P. zeylanica. C. albicans* was found to be sensitive to the hydroethanolic extract of *P. zeylanica* roots. Shukla et al. (2021) reported that extracts of *P. zeylanica* were active on a large number of microscopic fungi, hence their increased uses in traditional medicine to combat skin and mucous membrane conditions in humans and domestic animals. This antimicrobial potential of *P. zeylanica* extracts was attributed by Singh et al. (2017), largely to phenolic compounds and naphthoquinones represented mainly in the roots and in lesser quantities in the other plant organs of species of the *Plumbago* genus.

The highest MIC is obtained in *Shigella* *spp* and *S. aureus* MRSA with the root extract and the lowest is obtained in *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. The hydroethanolic extract of roots of *P. zeylanica* is found to be bactericidal on *Shigella spp*, *S. aureus* MRSA, *E. coli* ESBL, *E coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and bacteriostatic on *P. aeruginosa*. The hydroethanolic leaves extract was bactericidal against *S. aureus* MRSA and *S. aureus* ATCC29213. The same extract had no effect against the other microorganisms in this study. These results obtained are comparable to those reported by Shukla et al. (2021). According to their results, *P. zeylanica* extracts was effective on Gram-negative bacteria, Gram-positive cocci including multidrug-resistant strains of *E. coli* and certain microscopic fungi. The bacteriostatic action of the hydroethanolic extract of *P. zeylanica* root against *P. aeruginosa* could be linked to the genetic variability present in this bacterium. Indeed, *P. aeruginosa* is recognized for its faculties of frequent mutations and rapid acquisition of extra-chromosomal resistance genes giving it flexibility of adaptation against antimicrobials (Pang et al., 2019; Sarkar, 2020). This work reveals the effectiveness of the hydroethanolic extract of *P. zeylanica* root compared to the hydroethanolic extract of leaves.

5. Conclusion

The present study has demonstrated the antimicrobial potential of *P. zeylanica* roots and leaves extracts on reference strains and clinical isolates responsible for microbial infections. Phytochemical screening revealed the presence of flavonoids, tannins, phenolic compounds, sterols and triterpenes, cardenolipid glycosides and saponosides in the two extracts studied. The extract of the roots, the most active, was found to be active against Gram-negative bacilli, Gram-positive Cocci and *C. albicans* whereas the hydroethanolic extract of the leaves inhibited only the growth of *S. aureus* strains. This antimicrobial potential could be attributed to the secondary metabolites present in the extracts analyzed. This work helped elucidate the use of *P. zeylanica* in traditional medicine to combat infectious diseases. However, further studies are required to highlight the innocuity of the roots extract of the plant, isolate and identify the bioactive molecules for the formulation of new antimicrobial drugs.

Consent AND Ethical approval

It is not applicable.

References

Agban, A., Hoekou, Y, Pissang, P, Tchacondo, T, & Batawila, K. (2020). Evaluation du potentiel antimicrobien et de la toxicité des extraits de *Jatropha multifida* Linn, (Euphorbiaceae). *Journal of Applied Biosciences*, 151 : 15550-15558. DOI : <https://doi.org/10.35759/JABs.151.4>

Bouyahya, A., Dakka, N, Et-Touys, A, Abrini, J, & Bakri, Y (2017). Medicinal plant products targeting quorum sensing for combating bacterial infections. *Asian Pacific Journal of Tropical Medicine*, 10(8): 729‑743. DOI: <https://doi.org/10.1016/j.apjtm.2017.07.021>

Chaouche, TM., Haddouchi, F, Zerhouni, K, & Sidi-Yakhlef, A (2016). Évaluation de l’activité antimicrobienne de différents extraits d’*Helichrysum stoechas* subsp. Rupestre. *Afrique science*, 12(3) : 144‑150. DOI : <https://doi.org/10.25518/0037-9565.5894>

Chibuzor, JV., Onyegbule, FA, Umeokoli, B, Oyedemi, BO, & Lucky, EI (2024). Preliminary Phytochemical, Antioxidative and Antibacterial Screening of Selected Medicinal Plants for Possible use in the Treatment of Bacterial Diseases. Journal of Pharmaceutical Research International, 36(1): 38-60.

Dos Santos, LA., Cayô, R, Valiatti, TB, Gales, AC, de Araújo, LFB, Rodrigues, FM, de Carvalho, TS, Vaz, MAB, & Campanharo, M (2024). Biodiversity of carbapenem-resistant bacteria in clinical samples from the Southwest Amazon region (Rondônia/Brazil). *Scientific Reports*, 14(1): 9383. DOI: <https://doi.org/10.1038/s41598-024-59733-w>

EL-Haoud, H., Boufellous, M, Berrani, A, Tazougart, H, & Bengueddour, R (2018). Screening phytochimique d’une plante médicinale : *Mentha spicata* L. *American Journal of Innovative Research and Applied Sciences*, **7**(4): 226-233.

Epelboin, L., Robert, J, Tsyrina‐Kouyoumdjian, E, Laouira, S, Meyssonnier, V, & Caumes, E (2015). High Rate of Multidrug-Resistant Gram-Negative Bacilli Carriage and Infection in Hospitalized Returning Travelers: A Cross-Sectional Cohort Study. *Journal of Travel Medicine*, 22(5):292-299. DOI: <https://doi.org/10.1111/jtm.12211>

Gan, M., Zhang, Y, Yan, G, Wang, Y, Lu, G, Wu, B, Chen, W, & Zhou, W (2024). Antimicrobial resistance prediction by clinical metagenomics in pediatric severe pneumonia patients. *Annals of Clinical Microbiology and Antimicrobials*, 23(1): 33. DOI: <https://doi.org/10.1186/s12941-024-00690-7>

Harborne J, B (1991). Phytochemical Methods: A guide to modern techniques of Plant analysis. Chapman and Hall, London; pp 228.

Hoekou, Y., Tchacondo, T, Gbogbo, K, Tchelougou, D, Pissang, P, Karou, SD, Ameyapoh, Y, & Komlan, B (2015). Antibacterial activities of three latex plants of *Asclepiadaceae* family used in traditional medicine in South Togo. *International Journal of Current Microbiology and Applied Sciences*, 4**(**5**)**: 882‑891. <http://www.ijcmas.com>

Kagnou,H., Simalou, O, Gneiny, W, Tchani, Sanvee, S, Agbékonyi, A, Toundou, O, & Kpegba, K (2020). Etude phytochimique et activité antioxydante comparatives des trois variétés de *Catharanthus roseus* (L.) G. Don. *International Journal of Biological and Chemical Sciences*, 14(6): 2352‑2361. DOI: <https://doi.org/10.4314/ijbcs.v14i6.33>

Lagnika, L., Amoussa, AMO, Adjileye, RAA, Laleye, A, & Sanni, A (2016). Antimicrobial, antioxidant, toxicity and phytochemical assessment of extracts from *Acmella uliginosa*, a leafy-vegetable consumed in Bénin, West Africa. *BMC Complementary and Alternative Medicine*, 16: 34. DOI: <https://doi.org/10.1186/s12906-016-1014-3>

Mignanwandé, Z., Yélignan, Hounkpatin, AS, Christian, Johnson, R, Anato, D, Hinnoutondji, Kpètèhoto, W, Olatoundé, & Amoussa, M (2020). Etudes ethnomédicinale, phytochimie et activité antioxydante de *Crateva adansonii* DC (Capparidaceae) dans les communes de Cotonou et de Dassa-Zoumè au Benin. *Journal of Animal and Plant Sciences*, 46(1): 8071‑8089. DOI: <https://doi.org/10.35759/JAnmPlSci.v46-1.2>

NCCLS. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. Sixth edition. Wayne, PA: NCCLS. NCCLS document no. M07-A6. 32 p.

Ouedraogo, AS., Jean, Pierre, H, Bañuls, AL, Ouédraogo, R, & Godreuil, S (2017). Emergence and spread of antibiotic resistance in West Africa: Contributing factors and threat assessment. *Médecine et Santé Tropicales*, 27(2): 147‑154. DOI: <https://doi.org/10.1684/mst.2017.0678>

Ouro-Djeri, H., Koudouvo, K, Esseh, K, Tchacondo, T, Komlan, B, Wateba, M, Ouro-Djeri, E, & Gbeassor M (2022). Ethnopharmacological study of plants used in extemporaneous phytomedicines preparation at Tomety-Kondji, nearby township to the National Parc of Togodo South of Togo. *International Journal of Biological and Chemical Sciences*, 16(3): 967‑991. DOI: <https://doi.org/10.4314/ijbcs.v16i3.7>

Pang, Z., Raudonis, R, Glick, B, Lin T-J, Cheng, Z (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. *Biotechnology Advances*, 37(1): 177‑192. DOI: <https://doi.org/10.1016/j.biotechadv.2018.11.013>

Sarkar, S (2020). Release mechanisms and molecular interactions of *Pseudomonas aeruginosa* extracellular DNA. *Applied Microbiology and Biotechnology*, 104(15): 6549‑6564. DOI: <https://doi.org/10.1007/s00253-020-10687-9>

Shukla, B., Saxena, S, Usmani, S, & Kushwaha, P (2021). Phytochemistry and pharmacological studies of *Plumbago zeylanica* L.: A medicinal plant review. *Clinical Phytoscience*, 7(1): 34. DOI: <https://doi.org/10.1186/s40816-021-00271-7>

Sina, H., Dramane, G, Tchekounou, P, Assogba, MF, Chabi-Sika, K, Boya, B, Socohou, A, Adjanohoun, A, Baba-Moussa, L (2021). Phytochemical composition and *in vitro* biological activities of *Morinda citrifolia* fruit juice. *Saudi Journal of Biological Sciences*, 28(2): 1331‑1335. DOI: <https://doi.org/10.1016/j.sjbs.2020.11.059>

Singh, MK., Pandey, A, Sawarkar, H, Gupta, A, Gidwani, B, Dhongade, H, & Tripathi, DK (2017). Methanolic Extract of *Plumbago Zeylanica* - A Remarkable Antibacterial Agent Against Many Human and Agricultural Pathogens. *Journal of Pharmacopuncture*, 20(1): 18‑22. DOI: <https://doi.org/10.3831/KPI.2017.20.004>

Sohrabi, M., Pirbonyeh, N, Naini, M, A, Rasekhi, A, Ayoub, A, Hashemizadeh, Z, & Shahcheraghi, F (2024). A challenging case of carbapenem resistant *Klebsiella pneumoniae* related pyogenic liver abscess with capsular polysaccharide hyperproduction: A case report. *BMC Infectious Diseases*, 24(1) : 433. DOI : <https://doi.org/10.1186/s12879-024-09314-z>

Tidiane, K., Ouattara, G, Monon, K, Touré, A. & Ouattara, K (2021). Etude Phytochimique et Activité Antioxydante des Extraits D’écorces de Tiges de *Vitellaria Paradoxa* C.F. Gaertn, Une Plante Médicinale Utilisée au Nord de la Côte d’Ivoire. *European Scientific Journal,* 17(34) : 241. DOI : <https://doi.org/10.19044/esj.2021.v17n34p241>

Vasseur, M. (2014). Détermination de nouvelles modalités d’utilisation des bêta-lactamines en médecine vétérinaire par des approches PK/PD en vue de la protection de la santé publique : implication de la taille de la charge bactérienne pathogène*.* Thèse de Doctorat, Université de Toulouse, Toulouse, p.239.

Zhu, Z., Zhao, S, & Wang, C (2022). Antibacterial, Antifungal, Antiviral, and Antiparasitic Activities of *Peganum harmala* and Its Ingredients: A Review. *Molecules*, 27(13): 4161. DOI : https://doi.org/10.3390/molecules27134161