Anthocyanin content, lipid peroxidation inhibition and anti-salmonellosis activity of *Vitelariaparadoxa*Gaertn and *Parkiabiglobosa* (Jacq.) Benth bark extracts.

ABSTRACT

In a context where antimicrobial resistance limits the effectiveness of conventional treatments, the study of knowledge and practices related to anti-ulcer plants appears essential for exploring new therapeutic avenues of natural origin. The objective of this study is to determine the total anthocyanin content, the lipid peroxidation inhibition capacity and to evaluate the antimicrobial activity of *Vitellaria-paradoxa*Gaertn. and*Parkiabiglobosa* (Jacq.) Benth stem bark extracts on salmonella strains. Salmonella spp isolated at the CNHU bacteriology unit from blood and stool samples. The results reported that total anthocyanins were only quantifiable in extracts of *V. paradoxa* bark, with high levels ranging from 31.17 ± 17.13 to 66.79 ± 8.34 mg/g of plant powder. Ethanolic extracts of *V. paradoxa* showed strong inhibition of lipid peroxidation (54.43–65.46%), significantly higher than that of *P.biglobosa* extracts (32.01–43.90%) and comparable or higher than that of ascorbic acid (38.56%), with the Vp50 and Vp97 extracts showing statistically higher activity than ascorbic acid. Sensitivity tests show that the vast majority of bacterial strains tested are highly sensitive to *V. paradoxa* and *P. biglobosa* bark extracts (≈85–95% and >90%, respectively), reflecting the strong overall antimicrobial activity of all extracts. MICs ranged from 3.125 mg/mL to 50 mg/mL, while MBCs ranged from 12.5 mg/mL to 50 mg/mL. Each *P. biglobosa* extract showed bactericidal activity with MBC/MIC ratios between 0.5 and 4. *V. paradoxa* bark extracts showed predominantly bactericidal effects, accounting for approximately 72 to over 95% of responses for most solvents, particularly ethanol and ethyl acetate extracts, reflecting strong lethal antimicrobial activity. In contrast, *P. biglobosa* bark extracts showed more variable profiles depending on the solvent, with a predominance of bactericidal effects for ethanol and acetone extracts, but a higher proportion of undetermined effects, particularly with highly polar solvents, indicating more heterogeneous antibacterial activity.

Keywords: Natural compound, biological activities, plant extracts, Benin

1. Introduction

Salmonella enterica is a major zoonotic disease transmitted via the faecal-oral route through animals and the environment, causing gastroenteritis and typhoid fever in humans (Silva et al., 2014). Its serovars are increasingly posing a persistent public health challenge due to the recurring phenomenon of antimicrobial resistance (Nagpala et al., 2025; Sarkodie-Addo et al., 2025; Ugbo et al., 2025).

We note with astonishment that AMR is proving to be more deadly than pathologies that have been decried throughout history, and recent statistics estimate that by 2050, it will have claimed the lives of 39 million people (Naghavi et al., 2024; Institute for Health Metrics and Evaluation, 2024). In 2019, antimicrobial resistance (AMR) was responsible for 4.95 million deaths, including 1.27 million directly attributable to bacterial AMR, with a particularly high mortality rate in sub-Saharan Africa (27.3 deaths per 100,000 inhabitants) (Murray et al., 2022).

Antimicrobial resistance (AMR) in *Salmonella* remains an urgent global health challenge, with an upward trend in resistance to key antibiotics such as fluoroquinolones, tetracyclines and beta-lactams observed in humans, animals, food and the environment (Wang et al., 2022; Wang et al., 2025, Lv et al., 2025). Recent data for 2025 indicate high rates of multidrug resistance (MDR) exceeding 50% in Asia, alongside increasing resistance to nalidixic acid in Salmonella enteritidis from poultry and non-susceptibility to ciprofloxacin in egg and poultry-related outbreaks (Song et al., 2025). WHO surveillance between 2018 and 2023 showed an increase in resistance in more than 40% of pathogen-antibiotic combinations monitored for non-typhoidal*Salmonella*, complicating the treatment of infections such as those of the urinary tract, gastrointestinal tract and bloodstream (World Health Organisation: WHO, 2025).

There is little recent specific data on *Salmonella* AMR in Benin at the end of 2025, but studies point to widespread resistance in the poultry and agropastoral sectors (Deguenon et al., 2019).

In this global context of increasing antimicrobial resistance in *Salmonella* spp., the search for natural alternatives is essential. *Vitellariaparadoxa* and *Parkiabiglobosa* are two African plants widely reported for their ethnopharmacological benefits, particularly in the treatment of gastrointestinal diseases such as stomach pain, ulcers and diarrhoea in traditional medicine systems, and their extracts have shown gastroprotective, antioxidant and anti-inflammatory activities in modern experimental models. (Compaoré et al., 2024; Dangnon et al., 2024; Saleh et al., 2021). The various biological activities described above, particularly antimicrobial activity, clearly demonstrate the therapeutic potential of *Vitellariaparadoxa* and Parkiabiglobosa, justifying their traditional use and supporting their interest as sources of alternative bioactive agents. (Compaoré et al., 2024; Dangnon et al., 2025).

Although screening data have been reported, most studies have focused on quantifying total polyphenols, total flavonoids and tannins. Specific anthocyanins (aglycones: cyanidin, delphinidin, pelargonidin, etc.) are rarely quantified, and even less so in bark.

However, the anthocyanin class of natural flavonoid pigments has been associated with multiple bioactive activities, including antimicrobial and anti-biofilm properties, with recent data highlighting their potential to interfere with biofilm formation and

quorum sensing systems in pathogenic bacteria (Jeyaraj et al., 2023). Furthermore, these compounds exert potent antioxidant and anti-inflammatory activities, contributing to the modulation of oxidative stress and inflammatory responses in various biological models (Lakshmikanthan et al., 2024; Sadowska-Bartosz&Bartosz, 2024). These effects make anthocyanins increasingly interesting for prophylactic and therapeutic applications in bacterial infections and inflammatory disorders, including potentially gastroprotective properties via the reduction of inflammation and tissue oxidation. Oxidative stress, marked by lipid peroxidation (LPO), plays a key role in inflammatory and infectious diseases (Al-Kufaishi& Al-Musawi, 2025). Several experimental studies have shown that anthocyanins have a significant ability to inhibit lipid peroxidation (LPO) in vitro, thanks to their ability to trap free radicals such as hydroxyl radicals (•OH) and superoxides (O_2•$^-$) (Sadowska-Bartosz&Bartosz, 2024). These antioxidant mechanisms rely on the transfer of electrons or hydrogen atoms from anthocyanins to reactive oxygen species, thereby reducing oxidative damage to membrane lipids. Several in vitro experimental models have recorded significant inhibitions of LPO (sometimes exceeding 60% depending on the compound and system used) (Sadowska-Bartosz&Bartosz, 2024).

This study quantitatively assesses the total anthocyanin content (TAC), lipid peroxidation (LPO) inhibition capacity and anti-bacterial activity against *salmonella* of extracts from the bark of *V. paradoxa* and *P. biglobosa*.

2. Material and Methods

2.1. Collection of plant material

The bark of *V. paradoxa* and *P. biglobosa* stems was collected in February 2023 in the village of Sèmèrè, Donga Department, in northern Benin (9°33'19.444"N, 1°22'5.992"W). These organs were dried at 20±2°C for 15 days at the Laboratory of Biology and Molecular Typing in Microbiology at the University of Abomey-Calavi (UAC) in Benin before being ground into powder.

2.2. Preparation of extract

Methanol, ethyl acetate, acetone, methanol +1%HCl and ethanol (50%, 70% and 97%) were used as extraction solvents. The powdered bark of *V. paradoxa* and *P. biglobosa* (50 g) was extracted by maceration according to the protocol described by

Phrompittayarat et al. (2007) with slight modifications. The dried plant material was macerated in 500 ml of solvent for 72 hours with stirring at room temperature and filtered through filter paper (Whatman No. 1). The filtrate obtained was evaporated in a rotary evaporator and dried in an oven at 40°C. The residue collected was stored for further analysis. Although all extracts obtained from different solvents were evaluated for their antibacterial activity, particular attention was paid to ethanol extracts due to their ability to effectively extract a wide range of bioactive secondary metabolites and their better biological acceptability. Thus, ethanol extracts were used for anthocyanin assay and lipid denaturation inhibition testing.

2.3. Determination of the Total Anthocyanin Content of *Vitelariaparadoxa*and *Parkiabiglobosa*stem bark extracts

The Total Anthocyanin Content of extracts of *V. paradoxa* and *P. biglobosa* stem bark was measured by the pH differential method presented by Lee et al. (2005) and used by Taghavi et al. (2022) with slight modification. Briefly, 0.4mL of extract were mixed thoroughly separately with 2.6mL of pH 1.0 (0.225 M potassium chloride buffer) in triplicate and 2.6mL of pH 4.5 (0.4 M sodium acetate buffer) and then incubated for 15 min at room temperature and centrifuged at 4°C and 7000 rpm for 15 min. The supernatant was then removed, and the absorbance was read at 520 and 700 nm with Helios Gamma UV-Visible Spectrophotometer (Thermo). The following formula (5) was used to calculate the anthocyanin concentration. TAC (A × V)/M

Where: A = (A520 nm − A700 nm) pH 1.0 − (A520 nm − A700 nm) pH 4.5; V = volume of extract (mL) and M = fresh mass of the sample (g).

2.4. Lipid peroxidation inhibition activity of *V. paradoxa* and *P. biglobosa* stem bark extract

The lipid peroxidation inhibition activity of the extract was performed according to the method of Vamanu and Nita (2012).

In short, 1 mL of fowl egg yolk emulsified with phosphate buffer (pH 7.4) to obtain a final concentration of 25 g/L was mixed with the dilution of sample and 100 µL of 1mM FeCl2. The mixture was incubated at 37∘C for 1 h before being treated with 0.5 mL of freshly prepared 15% trichloroacetic acid (TCA) and 1.0 mL of 1% thiobarbituric acid (TBA). The reaction tubes were further incubated in a boiling water

bath for 10 min. Once cooled to room temperature, the assay tubes were centrifuged at 3500 g for 10 min to remove precipitated protein. The absorbance at 532 nm was determined spectrophotometrically (Helios Gamma UV-Visible Spectrophotometer (Thermo)). Ascorbic acid was used as standard. The percentage of inhibition (I%) was calculated from the following equation (12):

$$\text{inhibition (I\%)} = [(AAbb - AAss)/AAbb] \times 100 \quad (12)$$

where: $AAbb$ is the absorbance of the blank without the extract or ascorbic acid and $AAss$ is the absorbance in the presence of the extract or ascorbic acid

2.5. Evaluation of the antibacterial activity of extracts from the bark of *Vitellariaparadoxa* and *Parkiabiglobosa*.

2.5.1. Acquisition, confirmation and purification of bacterial strains.

A total of 22 clinical strains of Salmonella spp. isolated at the CNHU bacteriology unit from blood and stool samples were obtained with the consent of patients suffering from gastro-duodenal ulcers and confirmed using the specific *Salmonella Shigella* Agar medium.

2.5.2. Susceptibility of *Salmonella* strains to some commonly used antibiotics

The Bauer and Kirby method recommended by the WHO (World Health Organisation) was used to assess antibiotic resistance (Hudzicki, 2009). It is based on diffusion from antibiotic-impregnated discs onto Mueller-Hinton agar previously seeded by flooding with the bacterial suspension. The seeded plates containing the antibiotic discs were incubated for 24 hours at 37°C. After incubation, the results were read by measuring the diameter of the inhibition zones around each antibiotic disc. The results were interpreted according to the standard published by the Antibiogram Committee of the French Society of Microbiology (SFM, 2024). The following antibiotics were tested: Ceftriaxone (30 µg), Augmentin (30 µg), Telekinetic (10 µg), Erythromycin (5 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Tetracycline (30 µg), Amoxicillin with Clavulanic Acid (30 µg).

2.5.3. Evaluation of the antibacterial activity of extracts from *V. paradoxa* and *P. biglobosa*

The evaluation of antimicrobial activity consisted firstly of testing the sensitivity of the extracts on 22 clinical strains of Salmonella spp isolated at the CNHU bacteriology unit from blood and stool samples. The second step involved determining the antibacterial parameters, namely the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) from an extract concentration of 20 mg/ml.

2.5.3.1. Susceptibility test

The Muller Hinton (MH) solid medium diffusion method described by Hudzicki (2009) was used to test the sensitivity of microbial strains to extracts of *V. paradoxa* and *P.biglobosa*. A bacterial pre-culture (1 colony in 1 mL of liquid Muller-Hinton) from the previous day was diluted to obtain a turbidity of 0.5 on the McFarland scale (i.e. 10^8 CFU/mL) and reduced to 10^6 CFU/mL in sterile distilled water. This bacterial suspension (1000 µL) was used to flood a Petri dish containing Mueller-Hinton agar medium (Bio Rad, France) (SFM, 2024). Using a punch, 6 mm diameter paper discs were made. The sterile discs were placed under aseptic conditions on plates previously flooded with the bacterial culture. Under aseptic conditions, 30 µL of the extract to be tested was inoculated onto the discs. For each extract, the experiment was duplicated and a negative control was performed with the solvent instead of the extract. The plates were then left for 15-30 min at room temperature before being incubated at 37 °C in an incubator for 24 h and 48 h (Adesokan et al., 2007). The inhibition diameters were measured using a graduated ruler (Doughari et al., 2007) after incubation times of 24 hours and 48 hours.

The sensitivity of the 22 strains to the different extracts was characterised according to the scale of sensitivity of microorganisms to extracts established by Ganfon et al., (2019) (Table 1).

Table 1: Standard used for reading the results of antibiogram tests on plant extracts (Ganfon et al., 2019)

Determination of the inhibition zone (Δ)	Degree of microbial susceptibility

Δ < 7 mm	Insensitive
7 mm ≤ Δ < 8 mm	Sensitive
8 mm ≤ Δ < 9 mm	Fairly sensitive
Δ ≥ 9 mm	Highly sensitive

2.5.3.2. Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined in this study using the liquid microdilution method (Semeniuc et al., 2018) with iodo-dinitro-tetrazolium (INT) as a cell viability indicator. 96-well plates (8 rows of 12 wells) were used. A range of concentrations (50 to 0.0977 mg/mL) of the extracts were tested on 22 clinical strains of Salmonella sp isolated at the CNHU bacteriology unit from blood and stool samples. One hundred µL of sterile distilled water was distributed across all wells (from wells 2 to 10) of the plate. Next, 100 µL of each extract at a concentration of 200 mg/mL was added to wells 1 and 2 of the plate. Successive 1:2 dilutions were then performed from well 2 to well 10, and 100 µL from the last well was discarded. In addition, 100 µL of bacterial inoculum (10^6 CFU/mL) was added to all wells 1 to 10. The plate was then covered and incubated at 37°C for approximately 18 hours. After incubation, 10 µL of iododinitrotetrazolium (INT) solution was added to the wells and returned to the incubator at 37°C for 30 minutes. The MIC corresponds to the first well in which no red/pink colouration is observed, starting from the last well.

2.5.3.3. Determination of the Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined based on the results obtained from the MIC determination. To do this, after identifying the MIC, using a loop, all the other wells starting from the MIC towards the high concentrations were seeded on Petri dishes containing MH agar medium. The dishes were examined after 24 hours of incubation at 37°C. Upon observation, the concentration of the extract where no bacterial growth was observed corresponded to the MBC (Moroh et al., 2008). The antimicrobial effect of the extracts was determined by calculating the MBC/MIC ratio. If the ratio is less than or equal to 4, the extract is said to be bactericidal, and if it is greater than 4, the extract is said to be bacteriostatic (Ouattara et al., 2017).

2.5.3.4. Data processing and statistical analysis

The data obtained were entered into an Excel spreadsheet. The average total anthocyanin content was calculated and expressed as a mean ± standard deviation. The lipid peroxidation inhibition assay data were processed using GraphPad Prism 10, and vertical bar graphs were produced using an ANOVA test coupled with Tukey's post-hoc test.

The antibacterial activity data were also analysed using GraphPad Prism 10 software, vertical and/or stacked bar graphs were produced for the resistance rates of the clinical *Salmonella* strains studied, and the inhibition diameters of the *V. paradoxa* and *P. biglobosa* extracts were expressed as mean ± standard deviation. The MBC/MIC ratio (MBC/MIC) was calculated to assess the bactericidal and bacteriostatic activity of the extracts on the different strains tested.

3. Results

3.1. Total Anthocyanin Content (TAC) of stem bark of *V. paradoxa*and *P. biglobosa*extracts

The total anthocyanin content was determined for the different extracts and the results obtained are presented in Table 2. Total anthocyanins were only quantifiable in the *V. paradoxa* bark extracts. The *V. paradoxa* extracts have a high content. In ascending order, Vp97, Vp50 and Vp70 contained 31.17 ± 17.13 mg/g plant powder, 50.65 ± 36.67 mg/g plant powder and 66.79 ± 8.34 mg/g plant powder, respectively.

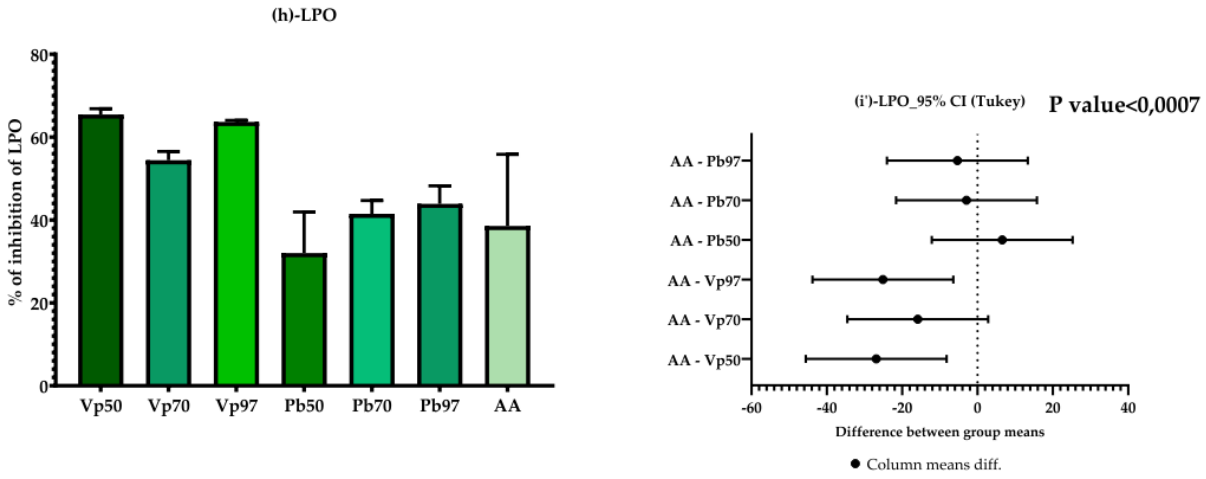
Table 2: Total anthocyanin content of the various extracts

Average TAC (mg/g of extract)					
Vp50	Vp70	Vp97	Pb50	Pb70	Pb97
50.65±36.67	66.79±8,34	31.17±17.13	<LOQ	<LOQ	<LOQ

LOQ: Limit of quantification

3.2. Lipid peroxidation (LPO) [(i); (l')] inhibition of ethanolic extract of stem bark of *V. paradoxa* and *P. biglobosa*

Figure 1 shows the results of the lipid peroxidation inhibitory activity of the ethanolic extracts of *V. paradoxa* and *P. biglobosa* bark. All ethanolic extracts (Vp50, Vp70 and Vp97) showed a lipid peroxidation inhibitory capacity of 65.46%, 54.43% and 63.68%, respectively, while the Pb50, Pb70 and Pb97 extracts showed lipid peroxidation inhibitory activity of 32.01%, 41.49% and 43.90%, respectively. The lipid peroxidation inhibition capacity of ascorbic acid was 38.56% (i). Tukey's one-way analysis of variance was used to compare the means and revealed that, with the exception of Vp50 and Vp97, which showed a statistically higher mean inhibition of lipid peroxidation than ascorbic acid, the inhibitory activity of the other extracts was statistically identical to that of ascorbic acid (i').



Vp: *Vitelaria paradoxa;* Pb: *Parkia biglobosa;* AA: Ascorbic acid; Degree at which ethanol where used for extraction (50; 70 and 97);
CI: Confidence Intervals

Figure 1: Lipid peroxidation (LPO) [(i); (l')] inhibition of ethanolic extract of stem bark of *V. paradoxa* and *P. biglobosa*

3.3. Prevalence of resistance in the Salmonella strains to some commonly used antibiotics.

Figure 2summarizes the results of the antibiogram test for Ampicillin (AMP2), Clindamycin (CD10), Gentamicin (CN10), Penicillin (P10), ceftriaxone (CTR10), tetracycline (TE30), streptomycin (S10), and norfloxacin (NX10) on the Salmonella strains in our study. These results showed that 100% of the strains are resistant to Ampicillin, Clindamycin and Penicillin, while 95.45% are resistant to Tetracycline. No

resistance was observed to Norfloxacin and Ceftriaxone. Furthermore, very low resistance was observed to Gentamicin (4.55%) and Streptomycin.

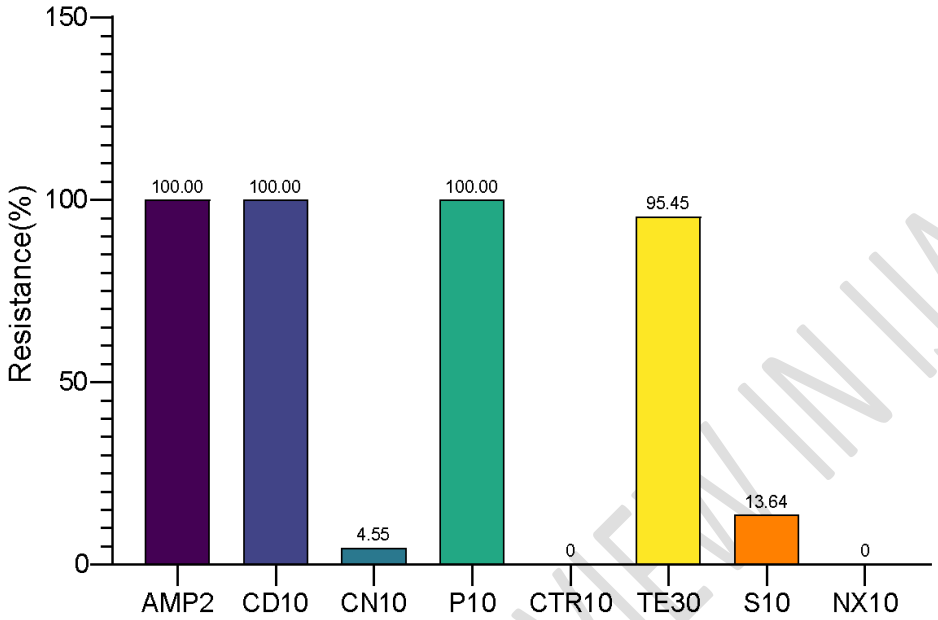


Figure 2 : Resistance status of the Salmonella strains studied to some commonly used antibiotics

3.4. Antibacterial activity of extracts from *V. paradoxa* and *P. biglobosa* on the *Salmonella* strains studied and on some reference strains

3.4.1. Susceptibility of bacterial strains to extracts of *V. paradoxa*and *P. biglobosa bark*

The results of sensitivity tests on bacterial strains tested with *V. paradoxa* bark extracts are shown in Figure 3. The figure shows the distribution of sensitivity levels (%) of the strains tested with regard to the different Vitellariaparadoxa extracts. In general, the 'Very sensitive' category dominates for all extracts, representing between 85 and 95% of responses. This indicates marked antimicrobial activity for all extracts studied. The ethyl acetate and acidified methanol extracts showed a slightly higher proportion of 'Fairly sensitive' strains, suggesting slightly less consistent efficacy compared to the other extracts. A few low percentages of 'Sensitive' and 'Insensitive' strains also appear, but sporadically (ethanolic extracts), confirming that resistance remains marginal for all extracts.

Methanolic extracts (VpMe, VpMeHCl) and ethyl acetate extracts (VpAcT) showed virtually no insensitive strains, demonstrating high and consistent efficacy. Approximately 4% of strains were insensitive to acetone and ethanol extracts.

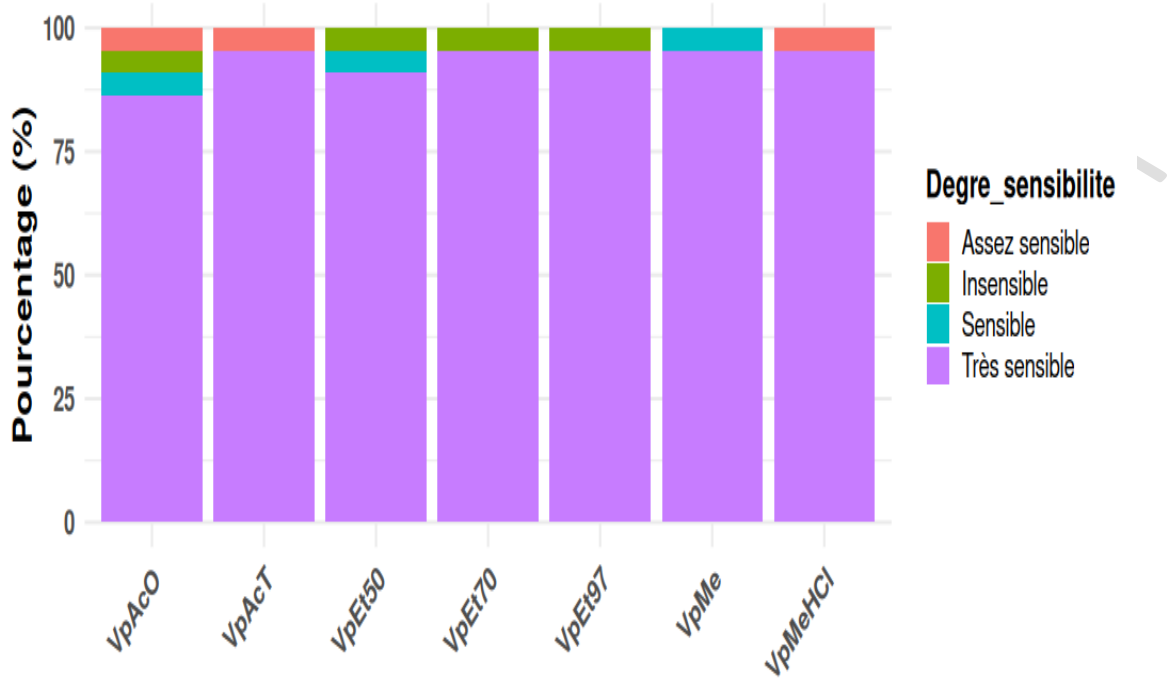


Figure 3: Inhibition diameters of *V. paradoxa*extracts on clinical *Salmonella* strains

The results of the bacterial strain inhibition test using P. biglobosa bark extracts are shown in Figure 4. The figure illustrates the distribution of sensitivity levels (%) of the tested strains to P. biglobosa extracts. It can be seen that, as with P. biglobosa, the 'Very sensitive' category dominates, generally accounting for more than 90% of responses for all extracts. This reveals a very marked and consistent antimicrobial activity of all *P. biglobosa* extracts. Some minor variations appear depending on the extract. The ethyl acetate (PbAcT) and acidified methanol extracts showed a slightly higher percentage of 'Fairly sensitive' and 'Sensitive' strains, indicating lower efficacy for a limited proportion of strains. The ethanolic extracts (PbEt50, PbEt70, PbEt97) and methanolic extract (PbMe) recorded a low proportion of 'sensitive' and 'insensitive' strains, which nevertheless remain marginal. Acetone extracts (PbAcO) showed very uniform activity, with an almost total predominance of 'very sensitive' strains. Overall, the variation between sensitivity categories remains very limited for *P. biglobosa* extracts.

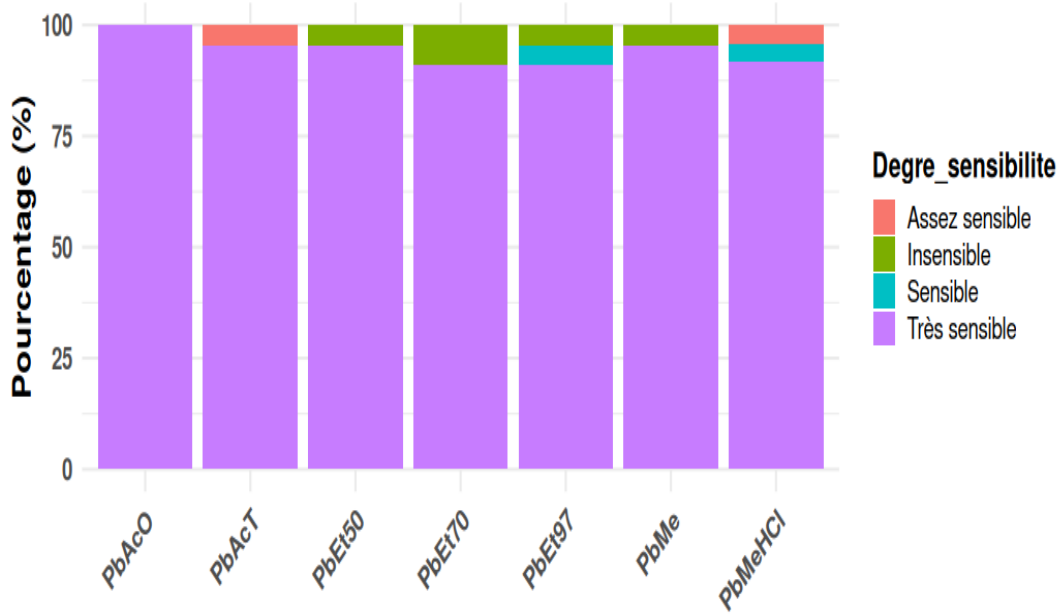


Figure 4 : Inhibition diameters of *P. biglobosa* extracts on clinical strains

3.4.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Table 3summarizes the minimum and bactericidal concentrations of *V. paradoxa* bark extracts. This table shows that, complementarily, the minimum inhibitory concentration and bactericidal concentration tests confirm the effectiveness of the antibacterial activity of *V. paradoxa* extracts. The extract based on acidified methanol (1% HCl) recorded lower pairs (MIC; MBC) on fewer strains (the *Salmonella* sp8 strain). The acetone extract recorded low pairs (MIC; MBC) on the most strains (13 different strains, including strains 4, 5, 6, 8, 9, 10, 12, 13, 14, 15, 17, 19 and 21).

Table 3: Minimum inhibitory concentrations(MIC) and Minimum Bactericidal Concentrations (MBC) of *V. paradoxa* extracts.

N° of strains	VpEt97		VpEt70		VpEt50		VpMe		VpMeHCl		VpAcO		VpAcT	
	MIC(mg/mL)	MBC (mg/mL)	MIC(mg/mL)	MBC (mg/mL)	MIC(mg/mL)	MBC (mg/mL)	MIC(mg/mL)	MBC (mg/mL)	MIC(m g/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)
1	25	25	25	>50	12.5	50	25	25	50	>50	25	25	6.25*	12.5*
2	25	50	12.5*	25*	12.5	50	12.5	50	50	>50	12.5	50	6.25	50
3	6.25*	25*	6.25*	25*	12.5*	25*	25	50	50	>50	12.5	25	25	25
4	25	25	25	25	12.5*	25*	6.25*	25*	50	>50	12.5*	25*	12.5	25

N° of strains	VpEt97		VpEt70		VpEt50		VpMe		VpMeHCl		VpAcO		VpAcT	
	MIC(mg/mL)	MBC (mg/mL)	MIC(mg/mL)	MBC (mg/mL)	MIC(mg/mL)	MBC (mg/mL)	MIC(mg/mL)	MBC (mg/mL)	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)
5	3.13*	12.5*	3.13*	12.5*	6.25*	12.5*	3.13*	12.5*	25	>50	1.56*	12.5*	3.13*	6.25*
6	25	50	25	50	50	50	25	25	25	>50	12.5*	12.5*	3.12*	6.25*
7	50	50	50	50	50	>50	50	50	50	>50	50	>50	50	>50
8	12.5	50	25	25	12.5	50	12.5	50	50	50	12.5*	25*	6.25*	25*
9	12.5*	25*	12.5	50	6.25*	12.5*	12.5*	25*	50	>50	12.5*	25*	12.5	50
10	12.5*	25*	12.5*	25*	12.5*	25*	6.13*	25*	50	>50	12.5*	25*	12.5	25
11	50	50	50	50	50	50	50	50	25	>50	50	50	6.25*	25*
12	25	25	50	50	50	>50	12.5*	25*	25	>50	6.25*	25*	6.25*	12.5*
13	3.13*	12.5*	6.25*	12.5*	12.5*	25*	3.13*	12.5*	50	>25	1.56*	12.5*	1.56	12.5
14	12.5*	25*	12.5*	25*	12.5*	25*	50	50	25	>25	12.5*	25*	6.25*	12.5*
15	50	50	50	50	50	50	50	50	25	>25	12.5*	12.5*	6.25*	6.25*
16	12.5*	25*	6.25*	25*	50	50	12.5	50	25	>25	3.13	50	3,13*	12.5*
17	12.5*	25*	6.25*	12.5*	25	>50	12.5	50	25	>25	6.25*	25*	12.5	25
18	50	>50	50	>50	50	>50	50	50	25	>25	50	50	25*	25*
19	25	25	12.5*	25*	12.5*	25*	12.5*	25*	25	>25	12.5*	25*	12.5*	25*
20	12.5	50	25	>50	12.5	50	25	25	25	>25	12.5	>50	12.5*	25*
21	50	50	50	>50	50	>50	50	>50	25	>25	0.78*	6.25*	1.56	12.5
22	3.125	>50	6.25	>50	6.25	>50	50	50	25	>25	25	50	25	50

*: Relatively low value

The minimum inhibitory and bactericidal concentrations of *P. biglobosa* bark extracts are summarized in Table 4. This table shows that each *P. biglobosa* bark extract recorded lower values (MIC; MBC) on at least one bacterial strain compared to the others. The methanolic extract with 1% HCl was the only one to record lower values on only two bacterial strains, *Salmonella* sp3 and 5. The 70% ethanol extract recorded very low values (MIC; MBC) on more strains (12 *Salmonella* strains). The 70% ethanol extract was effective against Salmonella sp1, 3, 5, 6, 8, 9, 10, 13, 14, 15, 16 and 17 strains.

Table 4: Minimum inhibitory concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of *P. biglobosa* extracts.

N° of strains	PbEt97		PbEt70		PbEt50		PbMe		PbMeHCl		PbAcO		PbAcT	
	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg/mL)	MIC(mg/mL)	MBC(mg /mL)	MIC(mg/mL)	MBC(mg/mL)
1	12.5	>50	12.5*	25*	12.5*	25*	12.5*	25*	25	>50	25	25	25	>50
2	50	>50	25	50	25	25	12.5	50	25	>50	12.5	50	25	>50
3	12.56 *	25*	12.5*	25*	6.25*	12.5*	6.25*	12.5*	12.5*	25*	12.5*	25*	12,5	>50
4	12.5*	25*	25	25	12.5*	25*	12.5*	25*	25	50	12.5*	25*	12,5	>50
5	6.25*	25*	0.39*	25*	0.39*	12.5*	0.78*	6.25*	6.25*	25*	1.56*	6.25*	6.25	>50
6	12.5*	25*	12.5*	25*	25	25	12.5*	25*	50	50	12.5*	25*	25	>50
7	50	>50	50	50	50	50	50	>50	50	50	50	>50	6.25	>50
8	50	>50	12.5*	25*	12.5*	25*	12.5*	25*	50	>50	12.5*	25*	6.25	>50
9	25	>50	12.5*	25*	12.5*	25*	25	25	10.25	50	12.5*	25*	6.25	>50
10	50	50	12.5*	25*	25	25	12.5*	25*	12,5	>50	12.5*	25*	12,5	>50
11	50	>50	50	50	50	50	50	50	6.25	>50	50	50	12,5	50
12	25	>50	25	>50	50	>50	25	>50	25	>50	12.5*	25*	12,5	>50
13	3.13*	12.5*	0.39*	12.5*	3.13*	12.5*	3.13*	25*	50	>25	12.5	50	12,5	>25
14	12.5*	25*	6.25*	12.5*	6.25*	25*	6.25*	12.5*	6.25	>25	12.5	>50	1,56	>25
15	12.5	>50	6.25*	25*	12.5*	25*	6.25*	25*	1,56	>25	12.5*	25*	1,56	>25
16	6.25	50	3.13*	12.5*	12.5	25	25	>50	25	>25	6.25	50	0,78*	1,56*
17	6.25*	25*	6.25*	25*	25	25	6.25	>50	25	25	12.5*	25*	6,25	>25
18	50	>50	50	>50	50	>50	50	50	25	50	50	>50	1,56*	3,13*
19	12.5	>50	12.5	>50	12.5	>50	12.5	>50	25	25	12.5*	25*	3,13*	3,13*
20	12.5*	25*	12.5	50	12.5*	25*	25	>50	25	>25	25	50	1,56*	12,5*
21	6.25	>50	25	50	12.5*	12.5*	50	>50	25	>25	1.56*	12.5*	1,56*	3,13*
22	12.5	>50	3.13	50	3.13	>50	25	>50	25	>25	1.56*	25*	1,56*	3,13*

*: Relatively low value

3.4.3. Characterisation of the activity of *V. paradoxa* and *P. biglobosa* bark extracts on the bacterial strains tested

Figure 5characterises the antibacterial activity of *V. paradoxa* bark extracts. The figure shows the percentage distribution of antibacterial effects observed for the different *Vitellariaparadoxa* bark extracts. Bactericidal and bacteriostatic effects are observed. However, these effects remain undetermined for certain strains. For most extracts (ethanolic, ethyl acetate, ethanolic and methanolic), bactericidal effects are the predominant category, ranging from approximately 72% to over 95%. This suggests strong lethal antimicrobial activity for most extracts, particularly those obtained with polar or semi-polar solvents. Bacteriostatic effects are present in only two extracts: acetone (18.2%) and ethyl acetate (13.6%). The effects remained undetermined on certain strains for the ethanolic and ethanol extracts (4.5% to

27.3%). The acidified methanol extract (VpMeHCl) stands out strongly with 95.5% undetermined effects and only 4.5% bactericidal effects recorded.

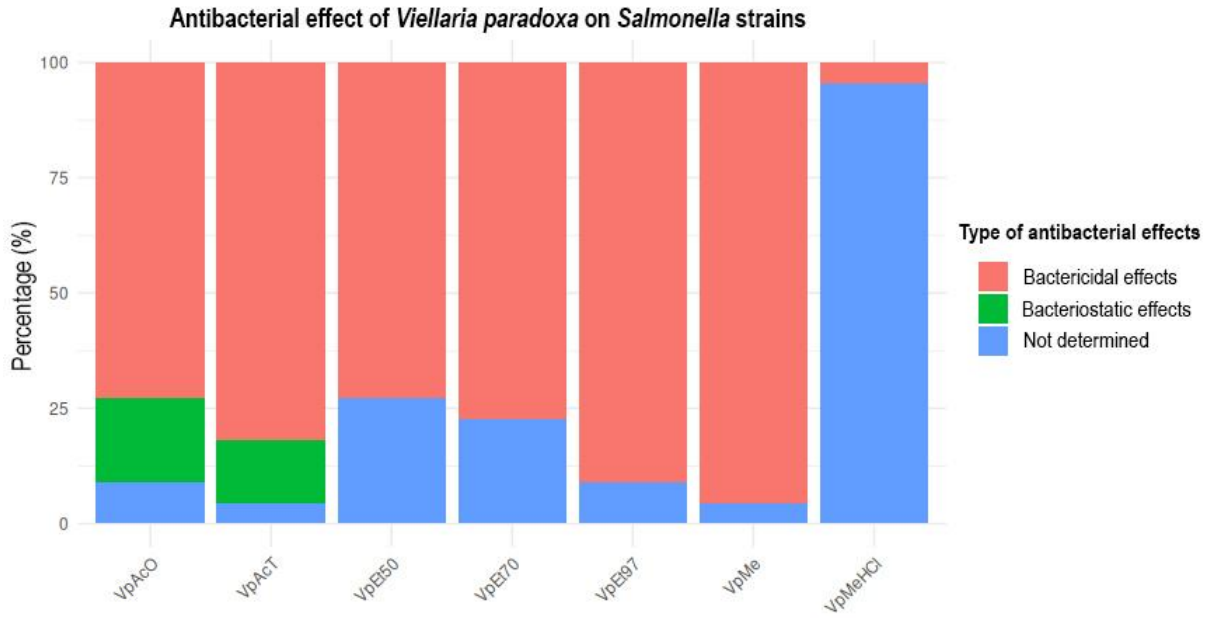


Figure 5: Bactericidal or bacteriostatic effects of *V. paradoxa* extracts on the strains studied.

The antibacterial effects of *P. biglobosa* bark extracts on strains are shown in Figure 6. The figure illustrates the percentage distribution of the antibacterial effects (bactericidal, bacteriostatic) of the different Parkiabiglobosa extracts. The profiles show marked variability depending on the solvents used. The majority of extracts show a significant proportion of bactericidal effects, ranging from 36.4% to 77.3%. The most bactericidal extracts are ethanolic (PbEt50 (77.3%) and PbEt70 (72.7%)) and acetonic (PbAcO (68.2%)). Bacteriostatic effects remain modest (4.5% to 18.2%) but are recorded for almost all extracts, including acetone (PbAcO (18.2%), ethanol (PbEt70 (13.6%)) and methanol (PbMe (9.1%)). The other extracts show low values (4.5%). Several extracts (ethyl acetate (PbAcT (68.2%)), methanol (PbMeHCl (59.1%)), and ethanol (PbEt97 (54.5%))) have a high proportion of undetermined effects. Highly polar solvents (97% ethanol, acidic methanol) show effects that are more difficult to classify.

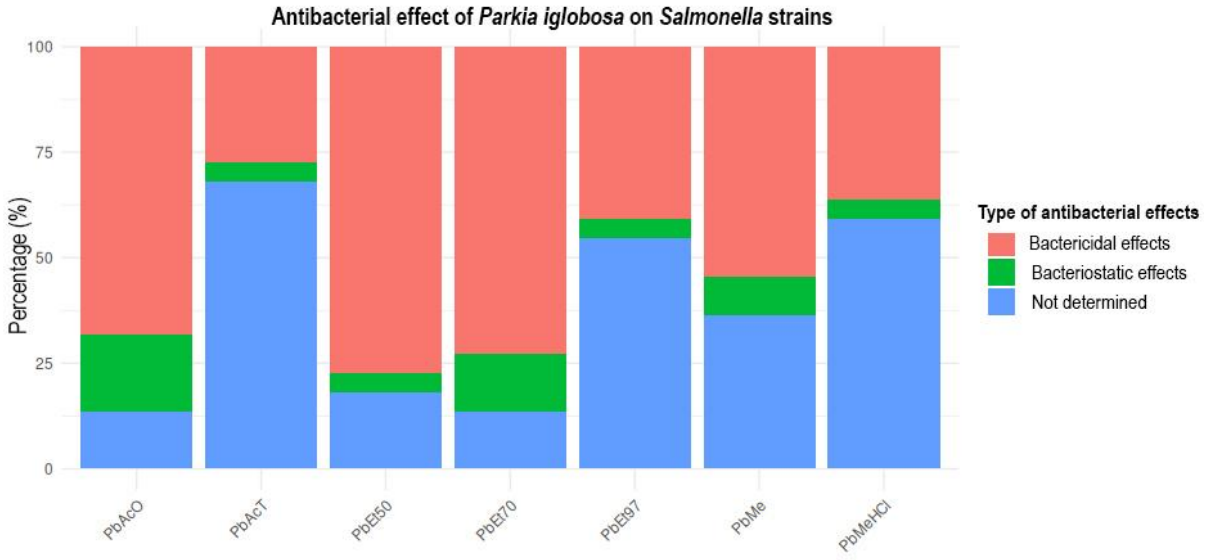


Figure 6 : Bactericidal or bacteriostatic effects of *P. biglobosa* extracts on the strains studied

Discussion

The determination of total anthocyanin content (TAC) revealed a very contrasting distribution between the two species studied. Anthocyanins were only quantifiable in extracts from the bark of *Vitellariaparadoxa*, while all extracts from *Parkiabiglobosa* had levels below the limit of quantification (<LOQ). This observation suggests a marked phytochemical specificity of *V. paradoxa* in anthocyanins, possibly linked to genetic, metabolic or anatomical differences between the two species, particularly in terms of flavonoid biosynthesis (Yan et al., 2021

Among the extracts of *V. paradoxa*, the anthocyanin content varied significantly depending on the hydroalcoholic degree of the extraction solvent. The Vp70 extract had the highest content (66.79 ± 8.34 mg/g of plant powder), followed by Vp50 (50.65 ± 36.67 mg/g) and Vp97 (31.17 ± 17.13 mg/g). This variation highlights the importance of solvent polarity in the extraction of anthocyanins, hydrophilic compounds known to be better solubilised in intermediate hydroalcoholic mixtures. The superior performance of 70% ethanol is consistent with data in the literature reporting optimal extraction of phenolic compounds in solvents of moderate polarity (Tourabi et al., 2025; Boeing et al., 2014). In terms of antioxidant activity, all V.

paradoxa extracts showed a strong ability to inhibit lipid peroxidation, with percentages ranging from 54.43% to 65.46%. In contrast, P. biglobosa extracts showed moderate inhibitory activity (32.01–43.90%), lower than that observed for V. paradoxa extracts. This interspecific difference suggests that the secondary metabolites present in greater quantities in *V. paradoxa*, particularly anthocyanins and other polyphenols, play a decisive role in protecting against lipid oxidation (Tidiane et al., 2021). This would be justified by the ability of anthocyanins to reduce the formation of lipid hydroperoxide, giving the extracts antioxidant potential (Klinger et al., 2024). Notably, the Vp50 and Vp97 extracts showed statistically superior lipid peroxidation inhibitory activity to that of ascorbic acid, used as a reference antioxidant. This result highlights the strong antioxidant potential of these extracts, possibly attributable to a synergistic effect between anthocyanins and other phenolic compounds such as flavonols, tannins or phenolic acids (Joshi et al., 2024). In contrast, the activity of Vp70, although high, was statistically comparable to that of ascorbic acid, suggesting that high anthocyanin content does not necessarily translate into proportionally higher antioxidant activity, highlighting the complexity of interactions between bioactive compounds (Joshi et al., 2022). These results further confirm the antioxidant potential of *V. paradoxa* and *P. biglobosa* bark extracts previously reported for the synthetic radicals DPPH, ABTS, FRAP and phosphomolybdate (Dangnon et al., 2025). Overall, these results indicate that V. paradoxa bark extracts are a promising source of natural antioxidant compounds, with efficacy sometimes superior to that of ascorbic acid. However, further studies, including structural identification of anthocyanins, evaluation of other phenolic classes and in vivo trials, would be necessary to better understand the underlying mechanisms of action and confirm their therapeutic potential.

The results of the antibiogram tests show a high prevalence of resistance to conventional antibiotics among the isolated *Salmonella* strains. Total resistance (100%) was observed to Ampicillin, Clindamycin and Penicillin, followed by almost total resistance (95.45%) to Tetracycline. These observations are consistent with numerous previous studies that report an alarming rise in resistance to commonly used antibiotics, particularly in resource-limited countries, due to their excessive or inappropriate use. In Nigeria, for example, multidrug resistance has been reported in Salmonella strains isolated from blood samples (Akinkunmi et al., 2023). The same

was true for strains isolated in Bangladesh from blood samples (Ghurnee et al., 2021; Mina et al., 2023). High levels of antimicrobial resistance have been reported among Gram-negative bacteria against commonly used antibiotics (Ombelet et al., 2022). In contrast, no resistance was noted to Norfloxacin and Ceftriaxone, while moderate or low resistance was noted to gentamicin (4.55%) and streptomycin, reflecting a certain residual efficacy of gentamicin and streptomycin and indicating that these, in addition to norfloxacin and ceftriaxone, remain among the therapeutic options that are still effective against these strains. Faced with this growing problem of resistance, the use of natural products with antimicrobial potential, such as plant extracts, is a promising alternative.

V. paradoxa bark extracts showed significant antibacterial activity against the strains tested, with inhibition zone diameters of up to 21.5 ± 3.5 mm. Ethanolic extracts (50% and 70%) proved to be particularly effective, as did methanol-based extracts with 1% HCl. The 70% ethanolic extract showed maximum activity against *S. aureus* ATCC29213, while the 50% extract stood out for its action against certain strains of Salmonella sp. The methanol extract with 1% HCl showed broad efficacy, inhibiting several strains with significant inhibition diameters, suggesting that acidification of methanol as an extraction solvent for *Vitellariaparadoxa* bark improves the extraction or release of antibacterial active ingredients. Bark and leaf extracts are reported to have antibacterial activity on clinical isolates of Bacillus cereus, *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli*, and *Salmonella typhi*. Compared to the leaves, the bark extract showed the highest activity with the largest inhibition zone of 15.5 mm (Lawrence et al., 2023). The largest inhibition diameter reported is well below the 21.5 ± 3.5 mm reported in our study for *V. paradoxa* bark. However, inhibition diameters of 18 to 24 mm have been reported for *V. paradoxa* bark extracts on *Serpulalacrymans*, *Sclerotiumrolfsii*, *Aspergillusfumigatus*, *Fomitopsispinicoca*, *Phaeolusschweinitzii*, *Rhizopus* spp., *Coniophoraputeana*, *Gloeophyllumsepiarium*, and *Fibroporiavaillantii* (Ekhuemelo et al., 2021). Furthermore, like *Salmonellatyphi* strains, *V. paradoxa* extracts inhibited the growth of *Burtholderiacepacia* and *Staphylococcusaureus* (Abdulazeez et al., 2023). The evaluation of minimum inhibitory concentrations (MIC) and bactericidal concentrations (MBC) confirmed the antibacterial activity of the extracts. In *V. paradoxa*, the majority of extracts had MBC/MIC ratios ≤ 4, which, according to the standard classification, indicates

bactericidal activity. The antibacterial molecules are believed to be distributed throughout the seeds of *V. paradoxa*, whose oil extract can induce inhibition ranging from 6 to 12 mm against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Streptococcus pyogenes*, and *Proteus mirabilis* with MICs and MBCs of 25 to 100 µg/ml and 50 to 100 µg/ml, respectively (Adegoke et al., 2024). The inhibitory and bactericidal concentrations reported in the microgram range are considerably low compared to those in the milligram range in our study.

P. biglobosa bark extracts also demonstrated antibacterial activity, with inhibition diameters of up to 21 ± 2 mm. As observed for *V. paradoxa*, ethanolic extracts at different concentrations, as well as methanolic and ethyl acetate extracts, were active against several *Salmonella* strains, with varying inhibition profiles. The 70% ethanol and methanol extracts each showed increased efficacy on a higher number of strains, demonstrating their broad spectrum of action. Ihuma et al. (2022) also reported antibacterial activity with inhibition diameters of 12.5 mm and 6.5 mm against *S. aureus* and *E. coli*, respectively. They recorded a higher MIC of 100 mg/ml (Ihuma et al., 2022), which is very high compared to the 50 mg/ml in our study. Similar results were observed in *P. biglobosa*. All extracts showed bactericidal activity on at least one strain, with MBC/MIC ratios ranging from 0.5 to 4. The 70% ethanol and methanol extracts were the most effective in terms of the number of sensitive strains with low MIC/MBC values. The methanolic extract with 1% HCl showed more limited activity, effectively inhibiting only one strain. The various organs of *P. biglobosa* are certainly reservoirs of antibacterial molecules. Indeed, the antibacterial activity of *P. biglobosa* fruit peel extracts has been reported in previous studies, particularly against *Pseudomonas aeruginosa* and *Escherichia coli*, with a minimum inhibitory concentration of 1.25 mg/mL (Bothon et al., 2023).

Overall, the extracts tested showed notable efficacy against multi-resistant strains of *Salmonella*. This observation supports their potential as alternative antibacterial agents. The mechanism of action of the extracts was not elucidated in this study, but it could involve disruption of the bacterial membrane, inhibition of protein or nucleic acid synthesis, or interference with cell communication (quorum sensing), which warrants further investigation. These results justify not only the pharmacological evaluation of *V. paradoxa* and *P. biglobosa*, but also the need for further chemical

characterization of the active fractions and assessment of their toxicity, bioavailability, and mechanisms of action.

Conclusion

This study highlights the significant antioxidant and antibacterial potential of extracts from the bark of *Vitellariaparadoxa* and *Parkiabiglobosa*, in a context marked by the worrying increase in antibiotic resistance. Phytochemical analysis revealed marked interspecific specificity, characterised by the exclusive and high presence of anthocyanins in *V. paradoxa* extracts, whereas these were not quantifiable in *P. biglobosa*. This particularity gives *V. paradoxa* a superior antioxidant capacity, reflected in a high inhibition of lipid peroxidation, sometimes superior to that of ascorbic acid. However, the absence of a strictly proportional correlation between anthocyanin content and antioxidant activity highlights the complexity of the synergistic interactions between the different phenolic metabolites. In terms of antibacterial activity, extracts from both species showed significant activity against multi-resistant strains of Salmonella. Extracts from *V. paradoxa* were particularly notable for their predominantly bactericidal profiles and high inhibition diameters, while those from *P. biglobosa* showed a broad spectrum of activity, depending on the extraction solvent. These results confirm the decisive role of extraction conditions in the release of active ingredients. Overall, these data support the pharmacological value of *V. paradoxa* and *P. biglobosa* as promising sources of natural antioxidant and antibacterial compounds. Nevertheless, further studies on the structural identification of bioactive compounds, the evaluation of their toxicity, bioavailability and mechanisms of action in vivo remain essential in order to confirm their therapeutic potential and future integration into alternative strategies for combating oxidative stress and antimicrobial resistance.

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