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Fruits of *Prosopis chilensis* and *Tetrapleura tetraptera* as an alternative against multi-resistant bacteria in lower respiratory tract infections

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ABSTRACT

Pseudomonas aeruginosa is a bacterium whose global spread poses a significant threat to human health due to its multidrug resistance (MDR). As a result, it is crucial to explore alternative treatments, particularly plant-based drugs, that are considered safe. The fruits of two plants, *Tetrapleura tetraptera*, and *Prosopis chilensis*, have been traditionally used to treat infectious diseases. These fruits are well-known for their nutritional and functional properties and their various bioactive compounds. Given these characteristics, the fruits can be effectively used against bacterial species like *P. aeruginosa*, which are resistant to conventional antibiotics. The present study aimed to evaluate the effects of fruit extracts on the multi-resistant bacterium *P. aeruginosa* PAO1. The research utilized methanolic, hydro-methanolic extracts, and aqueous decoctions of the selected fruits for phytochemical analysis and to assess antioxidant and antibacterial activities, along with acute toxicity. The study employed the 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) methods to examine antioxidant properties. The antibacterial activity was assessed through minimum inhibitory concentration (MIC), minimum biofilm concentration (BMC), and biofilm formation analysis. The results indicated that the methanolic extracts of *P. chilensis* and the aqueous decoction of *T. tetraptera* exhibited high total phenolic contents (135 and 143 mg GAE/g, respectively) and demonstrated the best antioxidant activity. Furthermore, the hydromethanolic extract of *T. tetraptera* showed the most substantial biofilm inhibition (70.15%) compared to the other extracts from both plants. Importantly, none of the extracts showed signs of toxicity at a dosage of 2000 mg/kg body weight. In conclusion, *T. tetraptera* and *P. chilensis* fruits contain compounds responsible for significant

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antioxidant activity and demonstrate efficacy against *P. aeruginosa* PAO1. These fruits could be potential candidates for developing phyto-drugs to combat antibacterial resistance in respiratory tract infections.

1 Introduction

According to the World Health Organization (WHO), an estimated 2.6 million deaths occur each year due to acute respiratory infections (ARI), with the majority occurring in developing countries (Mengistu et al. 2024). Additionally, Ghosh et al. (2023) indicated that ARI is the second leading cause of death globally, particularly among children. In the United States, pneumonia and bronchopneumonia account for 70 to 80% of admissions to healthcare facilities (Wood and Kuzel 2023). In Burkina Faso, roughly 23% of children aged 0 to 5 years exhibit symptoms of acute respiratory infection and fever (INSD 2022).

A common symptom of ARI is a cough, which may be accompanied by shortness of breath, weakness, fever, and fatigue. Coughing is particularly prevalent among the elderly, children under five, and immunocompromised individuals. The primary causes of these infections are often viral and/or bacterial (Kaler et al. 2023). The increased use of antibiotics and the application of incorrect dosages have led to the emergence of resistant strains of bacteria (Salam et al. 2023).

In response to the growing resistance of bacteria to the latest generation of antibiotics, medicinal plants may be effective alternatives in combating multidrug-resistant infections (Kang et al. 2023). The fruits of *T. tetraptera* are commonly utilized as a source of energy and nutrients, particularly for medicinal purposes (Kuate et al. 2015). Similarly, the fruit of *P. chilensis* is used for animal feed and in traditional medicine for its antimicrobial properties (Lorenzo et al. 2022).

This study is based on the hypothesis that these two fruits contain antimicrobial compounds that could serve as plant-based antibiotics against multidrug-resistant bacteria responsible for severe infections. The objective of this study was to evaluate the effects of methanolic, hydro-methanolic extracts, and aqueous decoctions of these two plants on the multidrug-resistant bacteria *Pseudomonas aeruginosa*, which is associated with lower respiratory tract infections (LRTI).

2 Materials and Methods

2.1 Microorganisms

The bacterial strain *Pseudomonas aeruginosa* PO1 was obtained from the Microbiology Laboratory of Joseph Ki-Zerbo University, Burkina Faso. Bacterial strain *P. aeruginosa* PAO1 was cultured in LB broth at 37 °C with agitation at 175 rpm. Its derivatives were grown in LB-MOPS broth (50 mM, pH 7), supplemented with carbenicillin at a concentration of 300 µg/mL, under the same temperature and agitation conditions.

2.2 Plant material

T. tetraptera and *P. chilensis* fruits were harvested from the Saaba commune in Burkina Faso. Specimens of *P. chilensis (Molina)* and *T. tetraptera* are deposited at the herbarium of Joseph KI-Zerbo University with code numbers 16741 and 16781, respectively.

2.3 Phytochemical characterization

The phytochemical characterization of the prepared plant extracts was conducted following the method described by Abubakar and Haque (2020). The dried material was powdered, stored in airtight containers, and kept in the dark at room temperature. Three solvents were used to extract compounds for biological activity testing: a 50:50 (v:v) mixture of water and methanol, methanol (98%), and water. The solvents of methanol and the methanol-water mixture were utilized to macerate the powdered fruit in a 1:10 (mass/volume) ratio. For the aqueous decoction, the mixture of water and material was boiled for 30 minutes.

2.4 Phytochemical assays

2.4.1 Assessment of total phenolic content

A microplate well is filled with 25 µL of the test solution (1 mg/mL), then combined with 125 µL of Folin-Ciocalteu reagent (FCR, 0.2). The mixture is incubated for 5 minutes. Next, 100 µL of sodium carbonate solution (7.5%) is added, and the incubation continues for 2 hours. Absorbance readings are taken at 760 nm. The results are expressed as milligrams of Gallic Acid Equivalent per gram of dry extract (mg GAE/g) (Singleton et al. 1999).

2.4.2 Assessment of total flavonoid content

The flavonoid content was determined using the method of Shraim et al. (2021). Absorbances were measured at 415 nm, and results are expressed as milligrams of quercetin equivalent (QE) per gram of dry extract (mg QE/g).

2.5 Biological activities

2.5.1 Antioxidant activity

The antioxidant activity was assessed using the DPPH method, as described by Velazquez et al. (2007). In this method, 100 µL of each sample (50 mg/mL) is mixed with 200 µL of DPPH solution (20 mg/L). This process is based on measuring the reduction in

absorbance at 515 nm of the free radical DPPH in the presence of hydrogen ions (H^+). The measured absorbance is then used to calculate the percentage inhibition of the DPPH radical, which reflects the antiradical potential of the sample. Ascorbic acid was used as a standard for comparison, and results were expressed in micrograms of ascorbic acid equivalent per 100 mg of extract (µg AAE/100 mg).

The evaluation of antioxidant activity using the FRAP method followed the procedure outlined by Hinneburg et al. (2006). In this method, 0.5 mL of the sample (0.625 mg/mL) is added successively to 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% potassium ferrocyanide. The mixture is then heated to 50°C for 30 minutes. After heating, 1.25 mL of 10% trichloroacetic acid is added, and the mixture is centrifuged for 10 minutes at 2000 rpm. From the resulting supernatant, 125 µL is transferred to wells in a 96-well plate, followed by the addition of 125 µL of distilled water and 25 µL of 1% ferric chloride. The absorbances are measured at 700 nm, and the results are expressed in millimoles of ascorbic acid equivalent (AAE) per gram of dry extract.

2.5.2 Determination of antibacterial activity

Liquid Luria-Bertani (LB) medium was prepared by dissolving 20 g of LB medium in 1 L of water with a pH of 7.2. The medium and materials were sterilized at 121°C for 15 minutes.

2.5.2.1 Determination of MIC (Minimum Inhibitory Concentration)

The extracts' minimum inhibitory concentrations (MICs) were determined using the 96-well microplate method (Barnes et al. 2023). The extracts were dissolved in 10% dimethyl sulfoxide (DMSO), and 20 µL of this solution was transferred into wells containing 170 µL of LB medium, resulting in a final DMSO concentration of 1%. A plain DMSO solution (1%) was used as a negative control.

Next, 10 µL of a *Pseudomonas aeruginosa* (PAO1) inoculum was introduced into each well, and the plates were incubated at 37°C for 18 hours. The MIC of the extract was identified as the first well that showed no bacterial growth. After the incubation period, 50 µL of p-iodonitrotetrazolium (INT) at a concentration of 0.2 g/mL was added to each well and incubated for 30 minutes. Microbial growth in the wells was indicated by the pink coloration of the INT (Jalal et al. 2023).

2.5.2.2 Determination of MBC (Minimum Bactericidal Concentration)

The Minimum Bactericidal Concentration (MBC) is the lowest concentration that can kill more than 99.9% of the initial bacterial population in the inoculum. Samples were collected from the control tube containing 1% DMSO and from each tube that showed no bacterial growth. These samples were then used to inoculate LB agar plates. The plates were incubated at 37°C for 24 hours. The MBC is determined from the first tube that exhibited no bacterial growth (Keyhanian et al. 2023).

2.5.2.3 Assessment of biofilm formation inhibition

Pseudomonas aeruginosa (PAO1) cells from an overnight culture were inoculated into 96-well plates containing 200 µL of growth medium, with or without extracts. After 18 hours of incubation, planktonic cells were removed, and adherent cells were fixed with methanol and stained with 0.2% crystal violet. After 5 minutes, the excess dye was washed away three times. Then, 33% acetic acid was added to each well, and the absorbance was recorded at 570 nm. Positive and negative controls, which contained only the inoculated growth medium and the growth medium with extract, were included in each experiment (Uppala et al. 2019).

2.6 Assessment of the acute toxicity

The toxicity of the extract was assessed following guideline 423 established by the Organization for Economic Co-operation and Development (OECD 2004) (Sasmito et al. 2017). Two groups of three female NMRI strain mice, weighing approximately 34 ± 3 g, were fasted for four hours before the experiment. The first group served as the control and received only plain water, while the test group was administered a dose of 2,000 mg/kg body weight of the extract via a feeding tube. The mice were observed continuously for two hours after administration and then regularly for the following fourteen days to monitor any signs of toxicity, which included tremors, convulsions, salivation, diarrhea, lethargy, sleep disturbances, and coma. The animals remained fast on the night of the fourteenth day and were subsequently sacrificed after being anesthetized. The liver, pancreas, and kidneys were collected from the treated and control groups to compare appearance and morphology. All animal experimentation protocols adhered to the guidelines of the Institutional Animal Ethics Committee (Directive 2010/63/EU on the protection of animals used for scientific purposes). Ethical approval code: 2010/63/EU, approval date: October 20, 2010.

2.7 Data processing and analysis

Calculations and graphs were performed using Microsoft Excel and GraphPad Prism software, version 5.0. Statistical analysis was conducted with IBM SPSS Statistics Base version 25. A significant difference is indicated by $p<0.05$.

3 Results

3.1 Total phenolic and flavonoid content

The total phenolic and flavonoid contents of the fruits from *P. chilensis* and *T. tetraptera* are summarized in Table 1. The study

Table 1 Evaluation of the total phenolic and flavonoid content in the selected plant extract

Plants	Extracts	Phytochemical characterization				Phytochemical dosage	
		Flavonoids	Tannins and Polyphenols	Alkaloid	Saponoside	Total phenolic (mg GAE/g extract)	Total flavonoids (mg QE/g extract)
P. chilensis	Methanolic	++	+++	+	++	143.72±13.02^{a}	10.46±3.04^{ac}
	Hydro-methanolic	++	++	+		232.45±0.39^{b}	31.22±2.64^{b}
	Aqueous decoction	+	++	+	++	38.99±2.57^{c}	14.22±0.52^{a}
T. tetraptera	Methanolic	++	++	+	++	87.94±0.81^{d}	9.43±0.55^{c}
	Hydro-methanolic	+	+	+	++	100.58±3.93^{d}	0.45±0.02^{d}
	Aqueous decoction	+	+	+	+	135.55±8.78^{a}	21.23±0.50^{e}

+ = presence; ++ = abundant; +++ = very abundant, QE: Quercetin Equivalent; GAE: Gallic acid equivalent; values in each column with differing superscript letters are significantly different ($P < 0.05$) for each phytochemical group measured

results showed that the total phenolic content in the samples ranged from 232.45 ± 0.39 mg GAE/g for the hydro-methanolic extract of *P. chilensis* to 38.99 ± 2.57 mg GAE/g for its aqueous decoction. For *T. tetraptera*, the total phenolic content of the hydro-methanolic extract was 100.58 ± 3.93 mg GAE/g. The findings indicated that the hydro-methanolic extract of *P. chilensis* contains many total phenolics and flavonoids. In contrast, in *T. tetraptera*, the highest concentrations of these compounds were found in the aqueous extracts. These observations suggest that hydro-methanolic solvents and water can extract total phenolics and flavonoids from *P. chilensis* and *T. tetraptera*.

3.2 Antioxidant activity

The FRAP and DPPH free radical scavenging activities of *P. chilensis* and *T. tetraptera* fruits are summarized in Table 2. The results indicate that all extracts exhibited similar activities when assessed using the FRAP method, regardless of the extraction solvent utilized. In contrast, according to the DPPH method, the methanolic and aqueous extracts of *P. chilensis* and *T. tetraptera* demonstrated the highest activity levels. The study revealed that the best antioxidant activities were recorded as 241.7 ± 0.52 mg AAE/g for the methanolic extract of *P. chilensis* and 271.35 ± 1.07

Table 2 FRAP and DDPH antioxidant activities of the tested fruit extract of *P. chilensis* and *T. tetraptera*

Plants	Extracts	DPPH activity (mg AAE/g)	FRAP activity (mmol AAE/g)
P. chilensis	Methanolic	241.7±0.52^{a}	18.34±1.66^{a}
	Hydro-methanolic	232.45±0.39^{b}	22.14±3.91^{ab}
	Aqueous decoction	228.25± 0.51^{a}	21.81±2.00^{ab}
T. tetraptera	Methanolic	263.25±12.69^{b}	19.64±1.37^{ab}
	Hydro-methanolic	256.85±2.57^{b}	20.07±1.21^{ab}
	Aqueous decoction	271.35±1.07^{b}	25.85±3.57^{b}

EAA: Ascorbic acid equivalent, values in each table column bearing different superscript letters differ significantly ($P < 0.05$) for each phytochemical group measured.

Table 3 Minimum inhibitory and bactericidal concentrations of extracts

Plants	Extracts	MIC (mg/ml)	BMC (mg/ml)	BMC/MIC
P. chilensis	Methanolic	12.5	>50	>1
	Hydromethanolic	50	>50	>4
	Aqueous decoction	50	>50	>1
T. tetraptera	Methanolic	-	-	-
	Hydromethanolic	-	-	-
	Aqueous decoction	-	-	-

-Inactive

mg AAE/g for the aqueous decoction of *T. tetraptera*, as measured by the DPPH method.

3.3 Antimicrobial properties of extracts

3.3.1 Determination of minimum inhibitory (MIC) and bactericidal concentrations (BMC)

Table 3 presents the minimum concentrations required to inhibit bacterial growth (minimum inhibitory concentration, MIC) and the bactericidal concentrations (BMC) where no bacterial growth was observed. The hydromethanolic extract of *P. chilensis* exhibited the lowest MIC of 12.5 mg/mL against *P. aeruginosa* PAO1, while all other extracts demonstrated MICs greater than 50 mg/mL. Additionally, the extracts of *T. tetraptera* showed no inhibitory or bactericidal activity against *P. aeruginosa* at a concentration of 50 mg/mL.

3.3.2 Inhibition of biofilm formation

Figure 1 illustrates the impact of various extracts on biofilm formation at a concentration of 100 µg/mL. The results indicated that the hydro-methanolic extract of *T. tetraptera* had the most significant effect, demonstrating an inhibition percentage of 70.15%. This was followed by the methanolic extract of the same plant, which showed an inhibition rate of 52.9%. Additionally, the methanolic extracts of *P. chilensis* and the aqueous extracts of *T. tetraptera* exhibited inhibition levels statistically similar to that of salicylic acid (48%), which served as the reference compound.

3.4 Acute toxicity of hydromethanolic extract

During the study period, observations of the animals revealed no signs of acute toxicity. A comparison of the organs between the test mice and the control group showed no differences. These results indicate that the extracts are not toxic at 2,000 mg/kg of body weight. Consequently, the LD50 was estimated to be 5,000 mg/kg of body weight, following the OECD guidelines.

4 Discussion

The total phenolic content of *T. tetraptera* extracts is higher than the 22.13 ± 0.14 mg GAE/g reported by Manga et al. (2020). However, the highest content found in the aqueous decoction of the same plant is lower than the 150.33 ± 0.036 mg GAE/g reported by Nwakiban et al. (2020). In contrast, the phenolic content of *P. chilensis* extracts exceeds the levels reported by Schmeda-Hirschmann et al. (2015). For *T. tetraptera*, studies have indicated a total phenolic content of 39 mg/g in the aqueous decoction of its fruits (Adadi and Kanwugu 2020), which is lower than our study's finding of 135 mg/g for the same extract. These discrepancies may be attributed to the type of reference compound used to create the standard curve; our study used gallic acid, whereas the referenced authors used ascorbic acid. Furthermore, the harvest period can also affect the levels of active ingredients in the tested extracts. Environmental factors and post-harvest conditions significantly influence the phytochemical composition of fruits (Delfanian et al. 2016). Regarding total flavonoid content,

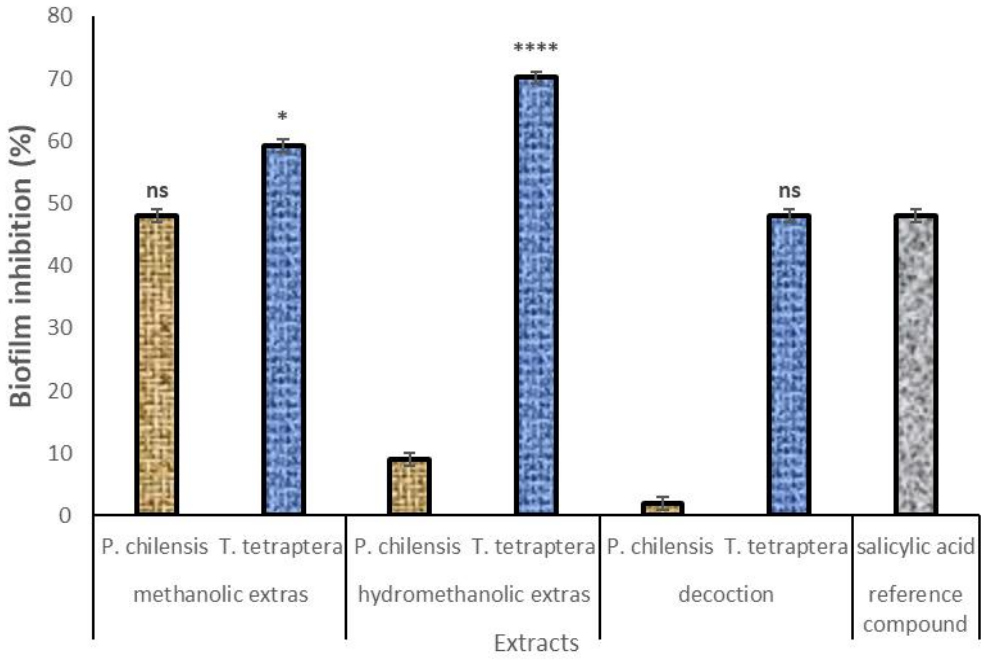

Figure 1 Inhibition of *P. aeruginosa* PAO1 biofilm formation by applying selected fruit extracts, ns: not significant compared with control (P< 0.05), *** significant difference

the hydromethanolic and methanolic extracts of *T. tetraptera* are higher than the 0.81 ± 0.03 mg QE/g reported by Adusei et al. (2019). In contrast, the decocted extracts of this same plant are lower than what Nwidu et al. (2019) reported. The flavonoid content of *P. chilensis* extracts exceeds the 5.6 ± 0.10 mg QE/g found by Schmeda-Hirschmann et al. (2015).

The findings indicate that water is the best solvent for extracting total phenolics from *T. tetraptera*. This fruit is commonly used to make juices and is added to tea preparations, creating an enriched source of phenolics. Overall, both aqueous and hydromethanolic extracts demonstrate a good phenolic content, suggesting that traditional extraction methods utilized by practitioners enable them to harness the medicinal properties of these compounds effectively. For *T. tetraptera*, the extract obtained through aqueous decoction exhibited the highest anti-free radical activity (DPPH), followed closely by the methanolic extract. The antiradical activity observed is greater than the 218.08 ± 1.20 mg AAE/g reported by Nwakiban et al. (2020). In the case of *P. chilensis*, the methanolic extract displayed the highest anti-free radical activity, followed by the hydromethanolic extract. The DPPH radical scavenging activity surpassed the value of 70.51 ± 0.01 mg AAE/g reported by Schmeda-Hirschmann et al. (2015).

The reducing properties of the extracts, as determined by the FRAP method, indicate that the hydromethanolic extract of *T. tetraptera* is superior to the values reported by Manga et al. (2020), which measured 1.11±0.38 mmol AAE/g.

The DPPH antiradical activity and the reducing properties (FRAP) of *T. tetraptera* extracts were higher than those of *P. chilensis* extracts. However, the total polyphenol and flavonoid content in *T. tetraptera* was lower than that in *P. chilensis*. These differences can be attributed to the type, quantity, and nature of phenolic compounds present in the extracts, such as rutin, luteolin, quercetin, ellagic acid, and catechin, which are known to be potent antioxidant molecules (Adadi and Kanwugu 2020).

The hydromethanolic extract of *P. chilensis* exhibited the lowest minimum inhibitory concentration (MIC) of 12.5 mg/mL. In this study, the minimum bactericidal concentration (MBC) to MIC ratio was greater than 4, indicating a non-bacteriostatic effect of the hydromethanolic extract of *P. chilensis* on *P. aeruginosa*. This effect may be explained by the multidrug resistance of *P. aeruginosa* PAO1 (Khan et al. 2020; Grace et al. 2022; Sebe et al. 2023). Furthermore, the extracts of *P. chilensis* at a concentration of 12.5 mg/mL did not exhibit bactericidal activity against *P. aeruginosa* PAO1. However, at a concentration of 100 µg/mL, these extracts significantly inhibited biofilm formation. Biofilms are adhesive and protective matrices synthesized by certain bacteria, contributing to their resistance (Dincer et al. 2020; Flemming and Wingender, 2010). This biofilm formation prevents antibiotics from effectively reaching the bacteria (Stewart 2002). The synthesis of biofilms is regulated by a communication system known as quorum sensing (QS), whereby bacterial cells communicate through signals carried by various molecules in their environment (Jiang et al. 2019).

The antibiofilm activity observed in the extracts suggests the presence of molecules that either inhibit QS by disrupting the biofilm or directly prevent its formation. On the other hand, *T. tetraptera* did not demonstrate significant antibacterial activity according to the tests used, but its extracts did inhibit biofilm production. Consuming *T. tetraptera* could reduce the risk of lower respiratory tract infections caused by resistant bacteria. Overall, the extracts of these fruits could complement external treatments in managing lower respiratory tract bacterial infections associated with biofilm formation and antibiotic resistance.

The findings of the toxicity study support the observations made by Bonsou et al. (2022). The absence of toxic signs induced by the extracts suggests that local populations regularly consume *T. tetraptera* as a beverage without risk. Given the high mortality rates linked to bacteria responsible for lower respiratory tract infections and the increasing resistance of these bacteria to antibiotics, many initiatives have focused on plant-derived medicines, with our study serving as an example.

Conclusion

Hydromethanolic, methanolic, and aqueous decoction extracts of *P. chilensis* and *T. tetraptera* fruits were used in this study. These fruits exhibited high levels of total polyphenols and total flavonoids. The extracts demonstrated significant activity against the resistant strain *P. aeruginosa* PAO1 by inhibiting biofilm formation. Additionally, *T. tetraptera* extracts are promising candidates for developing an antimicrobial phyto-drug effective against *P. aeruginosa* PAO1 due to their high efficacy and safety profile in mice.

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Conflict of interest

Authors declare no conflict of interest.

Ethical Declaration

The authors utilized animal models and adhered strictly to the ethical guidelines set by the institutional review board**.**

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