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Phytochemical analysis and antibacterial properties of *Terminalia phanerophlebia* and *Terminalia sambesiaca* leaf extracts

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ABSTRACT

Plants of the genus *Terminalia* are used in multiple traditional medicine systems globally, including in southern Africa. Whilst scientific studies have confirmed the therapeutic properties of some *Terminalia* species, other species remain relatively overlooked. This study examines the antibacterial properties of *Terminalia phanerophlebia* Engl. & Diels (TP) and *Terminalia sambesiaca* Engl. & Diels (TS) leaf extracts, and their ability to potentiate selected conventional antibiotics against antibiotic-sensitive and antibiotic-resistant pathogens. The antibacterial activities of the extracts and reference antibiotics were screened using disc diffusion assays and quantified using liquid microdilution MIC assays. The methanolic and aqueous TP and TS leaf extracts inhibited the growth of *S. aureus*, *E. coli* and *K. pneumoniae*, including MRSA and ESBL antibiotic-resistant strains, producing zones of inhibition on agar of 13–15 mm, and minimum inhibitory concentration values of 182–1500 µg/mL in most cases. Notably, the extracts inhibited MRSA growth with a similar efficacy to that observed against the susceptible *S. aureus* strain (133–265 µg/mL). Combining the extracts with tetracycline and chloramphenicol had substantially greater antibacterial activity than for either component alone, indicating that extract components block bacterial resistance mechanisms. In contrast, the extracts antagonised the activity of ciprofloxacin against some of the bacterial species. Extract toxicities were assessed using *Artemia* nauplii toxicity bioassays and were all found to be nontoxic. Additionally, Orbitrap LC-MS was used to identify and highlight noteworthy compounds in the extracts. Non-targeted LC-MS analysis revealed the presence of several noteworthy compounds, including tannins and flavonoids. Our findings suggest that the TP and TS extracts show promise for antibacterial drug development. Further study is warranted to elucidate their antibacterial and potentiating mechanisms.

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1. Introduction

The development of multiple drug resistant pathogens represents a significant health concern that has been acknowledged by organisations such as the World Health Organisation (WHO), the United States Centre for Disease Control (CDC), and the European Centre for Disease Control (ECDC) (World Health Organisation 2023; Elder et al., 2020). This problem is increasing in prevalence and severity. The primary driver behind the development of antimicrobial resistance (AMR) observed in hospitals, veterinary settings, and the wider community is the over-prescription and misuse of antibiotics (Memariani et al., 2019; Mittal et al., 2020). However, there is increasing evidence that antibiotics present in the environment due to anthropogenic

activities also contribute to the spread and development of AMR (Michael et al., 2013).

The term “superbug” has been attributed to AMR to define highly resistant pathogens that are largely unresponsive to conventional antimicrobial drugs (Algammal et al., 2023). The rapidly emerging threat of superbugs to global health is primarily driven by multi-drug resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacteria (World Health Organisation 2023). Among these, the six key pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) are known for their high levels of resistance to multiple classes of antibiotics (Aloke and Achilonu, 2023). These strains continue to plague hospital settings globally (Roch et al., 2023). The widespread use of antibiotic drugs has exerted selective pressure, driving these microorganisms to evolve and develop novel mechanisms of antibiotic resistance, as well as facilitating the transfer of genetic material between pathogens (Darby et

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al., 2023). This significantly limits the number of effective antibiotics and hinders the treatment of severe infections caused by these superbugs.

Numerous reports have explored traditional and herbal plant-derived antimicrobial therapies for their ability to potentiate the activities of current antibiotics for the treatment of AMR pathogens (Cheesman et al., 2017; Gideon and Ladan, 2023). South Africa, known for its rich floral biodiversity, relies heavily on preparations from plants as a primary healthcare approach for a significant portion of its population (Van Wyk and Prinsloo, 2018; Ndhlovu et al., 2021). The genus *Terminalia* (family *Combretaceae*) is widely used in several traditional healing systems in southern Africa, and the use of some species has been validated experimentally (Cock, 2015). Indeed, the antibacterial activity of multiple *Terminalia* spp. have been validated experimentally (Daniel et al., 2022; Nel et al., 2020), including against antibiotic-resistance pathogens (Zai et al., 2022; Tiwana et al., 2020; Zai et al., 2024). *Terminalia phanerophlebia* Engl. & Diels, commonly known as the Lebombo cluster leaf (Mposula et al., 2021), is distributed along the eastern seaboard of southern Africa. As it is a rich source of active metabolites, it is used medicinally in several tribal communities, particularly the Zulu ethnic group (Hutchings, 1996). It is used for its inflammatory, antibacterial and anti-septic properties in wound management, and to treat aches and pains, acne, sexually transmitted diseases and tuberculosis (Nair et al., 2012; Mposula et al., 2021). *Terminalia sambesiaca* Engl. & Diels is widely distributed throughout western and southern Africa (Masoko et al., 2005). Interestingly, this species has antimycobacterial properties and therefore has potential for the treatment of tuberculosis (Fyhrquist et al., 2014). However, the medicinal potential of both *T. sambesiaca* (TS) and *T. phanerophlebia* (TP) has been relatively neglected and substantially more study is required to rigorously assess their ability to inhibit the growth of bacterial pathogens.

This study examines the antibacterial effects of TP and TS extracts against *S. aureus*, *E. coli* and *K. pneumoniae*, as well as against the resistant counterparts of these bacterial species (MRSA, ESBL *E. coli* and ESBL *K. pneumoniae*). Liquid chromatography-mass spectrometry (LC-MS) was used to identify specific compounds present in the extracts that may be responsible for their antibacterial activity. The toxicities of the extracts were also measured using an *Artemia* nauplii (brine shrimp) lethality assay.

2. Methods

2.1. Plant sources

Dried TP and TS leaf materials were sourced from Lowlands Botanical Gardens, Nelspruit, South Africa and the identity was confirmed by Rejoice Mathebula of the gardens botanical staff. The leaf material was stored at the School of Environment and Science, Griffith University (QLD, Australia) as voucher numbers NBG-TP0719GU and NBG-TS0719GU, respectively.

2.2. Plant extract preparation

The dried leaf powders were weighed (1 g) separately into individual Greiner polypropylene tubes (Interpath Services Pty Ltd., Australia). Sterile deionised water or AR grade methanol (ChemSupply, Australia) were added to give a volume of 50 mL. The tubes were mixed for 20–24 h by rolling at 30 rpm at room temperature. Vacuum filtration through Whatman No 1 filter paper was used to obtain the filtrates. The methanol was subsequently air-dried at 42 °C in a chemical flow hood for 2 days. Aqueous extracts were freeze-dried by lyophilisation for 3 days in an Alpha 1-4 LSCplus benchtop freeze dryer (Martin Christ, Germany). The resultant crude extracts were weighed and then resuspended in water supplemented with 1 % dimethyl sulfoxide (DMSO; Merck, Australia) and sonicated three

times using 20 s pulses at 2 kHz. Finally, the resuspensions were sterilised by passing through 0.22 μ m filters (Sarstedt, Australia) and stored as 1 mL aliquots at –20 °C until required.

2.3. Antibiotics and bacterial species

Powdered antibiotics, including penicillin-G (potency of 1440–1680 μ g/mg), erythromycin (potency $\geq$ 850 μ g/mg), tetracycline ($\geq$ 95 % purity by HPLC), chloramphenicol ($\geq$ 98 % purity by HPLC), and ciprofloxacin ($\geq$ 98 % purity by HPLC) were purchased from Sigma-Aldrich (Australia) and used to prepare 1 mg/mL stock solutions for the broth microdilution assays. Discs containing penicillin (10 IU), erythromycin (10 μ g), tetracycline (30 μ g), chloramphenicol (30 μ g) or ciprofloxacin (1 μ g) were acquired from Oxoid Ltd. (Australia) and used in agar disc diffusion studies.

Escherichia coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), MRSA (ATCC 43300), *Klebsiella pneumoniae* (ATCC 13883), and ESBL *Klebsiella pneumoniae* (ATCC 700603) were obtained from the American Type Culture Collection (ATCC, USA). A wild-type clinical strain of ESBL *E. coli* strain was obtained from Gold Coast University Hospital, Southport, QLD, Australia.

2.4. Bacterial subcultures

Bacteria were grown using Mueller–Hinton (MH) agar and broth (Oxoid Ltd., Australia). All bacteria were cultured at 37 °C except for MRSA, which was cultured at 35 °C to avoid heteroresistance (growth of both susceptible and resistant staphylococci), which may arise in MRSA when cultured at temperatures above 35 °C (Thornsberry et al., 1973). Prior to the antibacterial assays, bacterial colonies were inoculated into MH broth and incubated for 16–18 h or until a cell count of approximately 10^8 cells/mL was reached.

2.5. Antibacterial assays

All antibacterial test conditions conformed to CLSI standardised methods (Clinical and Laboratory Standards Institute, 2019). MH agar was used for the disc diffusion assays (Tiwana et al., 2020). Discs containing antibiotics or undiluted extracts were affixed to the agar, which had been spread with 100 μ L of individual log phase bacterial suspensions. Following overnight incubation, inhibition of bacterial growth surrounding the discs was measured to the nearest whole millimeter and are reported as the zone of inhibition (ZOI) for each disc. Samples that failed to inhibit growth were reported as having a ZOI of 6 mm (the diameter of the filter discs applied to the agar surface). Liquid dilution broth assays were used to determine MIC values for all extracts and antibiotics as previously described (Ilanko and Cock, 2019). Extract MIC values >5000 μ g/mL were considered inactive; MIC values between 2000 and 5000 μ g/mL were considered as low activity; 1000–2000 μ g/mL were considered as moderate activity; 400–1000 μ g/mL were considered as noteworthy activity; 100–400 μ g/mL were considered as good activity; and <100 μ g/mL were considered to be high activity (Elisha et al., 2017).

2.6. Fractional inhibitory concentration (FIC) and Σ FIC determinations

Extracts that inhibited bacterial growth in the microdilution broth assay were further studied in combination with selected conventional antibiotics to determine the class of interactions between the components. Antibiotic and extract samples were mixed at a 50:50 ratio, and 100 μ L of this solution was tested in this assay alongside control lanes containing extract alone, antibiotic alone, as well as broth and solvent controls. Interactions between the plant extracts and antibiotics were measured through the sum of fractional inhibitory concentrations (Σ FIC) for each combination studied. This interaction was determined through the calculation of each component (A

and B) in the 50:50 ratio using the following equations, where A represents the extract sample, and B represents the antibiotic.

$$FIC(A) = MIC(A \text{ in combination with } B) / MIC(A \text{ independently})$$

$$FIC(B) = MIC(B \text{ in combination with } A) / MIC(B \text{ independently})$$

The correlation between the extract (A) and antibiotic (B) components was determined using the equation $\Sigma FIC = FIC(A) + FIC(B)$. The interactions were classified as synergistic ($\Sigma FIC \leq 0.5$), additive ($\Sigma FIC > 0.5 - \leq 1.0$), indifferent ($\Sigma FIC > 1.0 - \leq 4.0$) or antagonistic ($\Sigma FIC > 4.0$) (Doern, 2014).

2.7. Phytochemical assays

Qualitative phytochemical assays for all extracts were conducted using standard methods to detect the presence of phenolic compounds, cardiac glycosides, saponins, triterpenoids, phytosterols, alkaloids, flavonoids, tannins and anthraquinones, along with approximations of their relative abundances (Vesoul and Cock, 2011).

2.8. LC–MS metabolomic analysis of the plant extracts

Orbitrap liquid chromatography–mass spectrometry (HPLC–MS) using previously developed parameters (Zai et al., 2023) was used to separate and identify compounds in the TP and TS leaf extracts. The Orbitrap Exploris 120 mass spectrometer utilised electrospray ionisation in negative ionisation mode. Individual compounds were identified by comparison of their retention index, molecular mass and mass spectral properties with ChemSpider, CyanoMetDB, Global Natural Product Social Molecular Networking (GNPS), mzCloud, mzVault and databases.

2.9. Toxicity assays

Toxicity screening of the extracts was conducted using *Artemia franciscana* Kellogg nauplii lethality assays (ALA) (Ruebhart et al., 2009; Shalom and Cock, 2018). LC_{50} values were calculated using probit analysis as the extract concentration required to kill 50 % of the *A. franciscana* nauplii.

2.10. Statistical analysis

Samples were tested in triplicate for disc diffusion assays, and statistical analysis was conducted, with the data expressed as the mean $\pm$ SEM of three independent experiments. Differences between control and treatment groups were analyzed using one-way analysis of variance (ANOVA), with p values < 0.05 considered to be statistically significant; p values < 0.01 as highly statistically significant; and p values < 0.001 considered to be very highly significant. All broth microdilution assays were repeated twice for each bacterial strain on separate days, with two technical replicates per assay ($n = 4$), to ensure the reproducibility of the MIC determinations.

3. Results

3.1. Qualitative phytochemical assays

Table 1 shows the extract yields and qualitative assessments of the abundances of several different phytochemical classes. The yields of all extracts were similar (23–35 mg/mL) and contained high levels of tannins, flavonoids, and water-soluble and insoluble phenols. Lower levels of saponins, triterpenoids and cardiac glycosides were detected in the extracts, while phytosterols, anthraquinones and alkaloids were not present.

Table 1
Concentrations of the plant extracts and their qualitative phytochemical analysis.

Extract type	Concentration ^a (mg/mL)	Phytochemical class and abundances										
		Total Phenolics	Water Soluble phenols	Water Insoluble phenols	Cardiac Glycosides	Saponins	Triterpenoids	Phytosterols	Alkaloids	Flavonoids	Tannins	Anthraquinones
TP-Aq	29.0	++	++	++	+	++	+	-	-	++	++	-
TP-MeOH	23.2	+++	+++	+++	+	+	+	-	-	+++	+++	-
TS-Aq	34.4	+++	+++	+++	++	+	+	-	-	+++	+++	-
TS-MeOH	24.4	+++	+++	+++	+	-	+	-	-	+++	+++	-

^a Values obtained following resuspension in 1 % DMSO. +++ indicates a large response in the assay, ++ indicates a moderate response, + indicates a minor response, - indicates no response. TP = *T. phanerophlebia*, TS = *T. sambesiaca*. Aq = aqueous, MeOH = methanol.

3.2. Disc diffusion assays

While the TS and TP extracts were inactive against *E. coli*, ESBL *E. coli*, *K. pneumoniae* and ESBL *K. pneumoniae* on agar, the aqueous and methanolic TP and TS extracts produced relatively large ZOI against *S. aureus* and MRSA (Fig. 1). These large ZOIs reached values of up to 15 mm for both species in some cases, indicating that the resistant strain (MRSA) was as susceptible to these extracts as was the sensitive (*S. aureus*) strain. The levels of inhibition between the aqueous and methanolic extracts of TP and TS were also similar for both bacteria, suggesting that both plant species have similar potencies against *S. aureus* and MRSA.

3.3. MIC assays

The MIC values for the antibiotics and the plant extracts as determined by the microdilution broth method are shown in Table 2. The aqueous and methanolic TP and TS extracts inhibited the growth of all bacterial species. Against ESBL *K. pneumoniae*, both plants showed low inhibitory activity, with the lowest measurable activity for the aqueous TP extract at 7250 $\mu\text{g/mL}$. Moderate activities were observed for both plants against *K. pneumoniae*, *E. coli* and ESBL *E. coli*, with similar MIC values of approximately 1500 $\mu\text{g/mL}$. An exception was noted for the TP and TS aqueous extracts against *K. pneumoniae*, which produced noteworthy MIC values of 453 $\mu\text{g/mL}$ and 531 $\mu\text{g/mL}$ respectively. The strongest activities were detected using the TP and TS extracts against the *S. aureus* strains. They exhibited good activities against both *S. aureus* and MRSA, with MIC values ranging from 133 to 265 $\mu\text{g/mL}$. These findings indicate that aqueous and methanolic TP and TS extracts are highly effective inhibitors of *S. aureus* and MRSA growth, while the aqueous extracts are effective inhibitors of *K. pneumoniae* growth, and to a lesser extent, *E. coli* growth in liquid culture.

While the majority of activities observed in the MIC assays were concordant with the agar disc diffusion assays, there are several notable exceptions involving the aqueous and methanolic TP and TS extracts. These extracts inhibited both sensitive and resistant *E. coli* and *K. pneumoniae* bacterial strains in liquid MIC assays, although they did not inhibit the growth of the corresponding bacteria on agar (Fig. 1; Table 2).

3.4. Combinational studies

Combinational studies were conducted to assess potential interactions between the antibiotics and extracts in this study. For these assays, both the extract and the antibiotic that were tested against a particular bacterial strain must have exhibited some level of activity, as MIC values are required to measure FIC values. Ultimately, the TP and TS extracts were tested in combination with at least one antibiotic against five of the six bacterial strains, and the FIC and ΣFIC values were determined in these cases. These results are shown in Tables 3–6.

Synergistic enhancement of antibacterial activity was not observed for any of the combinations tested. However, additive, indifferent, and antagonistic interactions were detected. Table 3 shows the outcomes of antibiotic combinations with the aqueous TP extract. Additive interactions (ΣFIC values >0.5 – ≤ 1.0) were observed for combinations containing tetracycline against *S. aureus*, MRSA and *E. coli*, and for combinations containing chloramphenicol against *E. coli*. Additionally, combinations containing ciprofloxacin produced additive effects against MRSA, while combinations of this extract with ciprofloxacin produced an antagonistic (ΣFIC values >4.0) interaction (Table 3). All other interactions in combinations containing this extract were indifferent (ΣFIC values >1.0 – ≤ 4.0), including combinations of aqueous TS extract with either erythromycin or

ciprofloxacin against *S. aureus*, and tetracycline or chloramphenicol against *K. pneumoniae*.

The combination of the methanolic TP extract and tetracycline produced additive interactions against *S. aureus*, MRSA, *E. coli*, and *K. pneumoniae*. Similarly, the combination of TP with chloramphenicol was additive against *E. coli* (Table 4). Several indifferent interactions were noted for combinations containing this extract, including combinations containing erythromycin or ciprofloxacin against *S. aureus*, or with ciprofloxacin against MRSA, with chloramphenicol against *K. pneumoniae*, or with ciprofloxacin against ESBL *K. pneumoniae*.

The aqueous TS extract was also tested in combination with conventional antibiotics against the bacterial panel. An additive interaction was observed for this extract in combination with tetracycline against both MRSA and *E. coli*. Similarly, the combination of the aqueous TS extract with chloramphenicol against *E. coli* was also additive, whilst the combination of the extract with ciprofloxacin was antagonistic against ESBL *K. pneumoniae* (Table 5). Indifferent interactions were observed for combinations of the extract with either erythromycin, tetracycline or chloramphenicol against *S. aureus*, as well as with ciprofloxacin against MRSA, and with tetracycline or chloramphenicol against *K. pneumoniae*.

The methanolic TS extract produced additive interactions when combined with tetracycline against *S. aureus*, MRSA, *E. coli* and *K. pneumoniae*, and when combined with chloramphenicol against *E. coli* (Table 6). Combination of the methanolic TP extract with ciprofloxacin produced an antagonistic interaction against ESBL *K. pneumoniae* growth inhibition. All other interactions with the TP extract were indifferent, including erythromycin or ciprofloxacin against *S. aureus*, or with ciprofloxacin against MRSA, or chloramphenicol against *K. pneumoniae*.

Two consistent trends were evident from these combinational studies involving the *Terminalia* spp. extracts. First was their ability of the extracts to interact with tetracycline and chloramphenicol to produce additive increases in antibacterial activity. Secondly, when three of the four *Terminalia* extracts (aqueous TP, as well as aqueous and methanolic TS extracts) were used, antagonism of growth inhibition was observed when the extracts were combined with ciprofloxacin.

3.5. *Artemia franciscana* Kellogg nauplii lethality assays (ALA)

The plant extracts were tested in triplicate on 48-well plates by ALA to measure levels of toxicity. Plant extracts were deemed to be toxic if LC_{50} values were <1000 $\mu\text{g/mL}$ after 24 h exposure (Ruebhart et al., 2009; Tiwana et al., 2020). All plant extracts tested in this study were deemed to be non-toxic as $>50\%$ of the nauplii remained alive following this incubation period. Indeed, all extracts produced results similar to the negative control (sea water) in which 100 % of the brine shrimp were viable after 24 h.

3.6. LC–MS identification of *T. phanerophlebia* and *T. sambesiaca* leaf compounds

Non-biased Orbitrap LC–MS metabolomics analysis using optimised parameters previously developed by our group (Zai et al., 2023) was used to putatively identify compounds in the *T. phanerophlebia* and *T. sambesiaca* leaf extracts (Table 7). The chromatograms for each of the extracts are shown in Supplementary Figures 1 and 2. In general, greater compound complexity was noted for the *T. phanerophlebia* extracts (74 and 98 unique mass signals for the methanolic and aqueous extracts respectively; unrepresented data), compared to the *T. sambesiaca* extracts (77 and 62 for the methanolic and aqueous extracts respectively). The retention indexes, molecular masses and spectral properties of all mass signals were compared to chemical and spectral databases. Matches were excluded and classified as unidentified compounds if their retention indexes were $\geq 5\%$

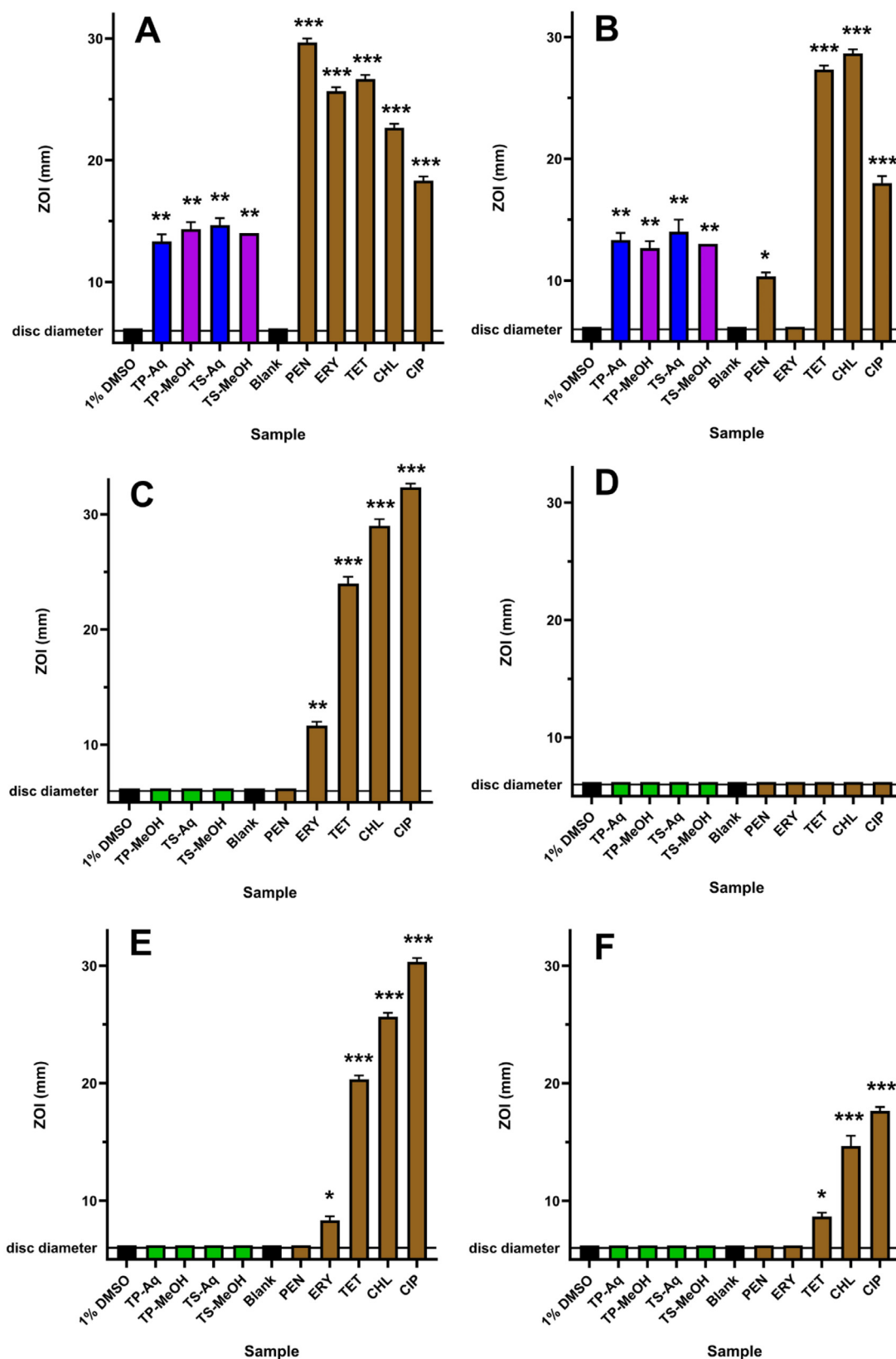


Fig. 1. Agar disc diffusion assays for plant extracts and reference antibiotics against (A) *S. aureus*, (B) MRSA, (C) *E. coli*(D), ESBL *E. coli*(E), *K. pneumoniae*, and (F) ESBL *K. pneumoniae*. The ZOI values were measured in mm and the disc sizes were 6 mm, as indicated on the y-axis. Negative control discs contained 1 % DMSO (for crude extracts) or blank discs (for antibiotics) and are shown by the black bars. Inactive extracts are in green, while antibiotics are shown as brown bars. Aqueous and methanolic extracts showing activity are shown by blue and purple bars, respectively. Each value is expressed as mean $\pm$ SEM of triplicate assays of each extract and antibiotic. Results are significantly different to the negative control (1 % DMSO for extracts; blank for antibiotics) if $p < 0.05$ (*), < 0.01 (**) or < 0.001 (***). TP = *T. phanerophlebia* leaves, TS = *T. sambesiaca* leaves, Aq = aqueous, MeOH = methanol, PEN = penicillin, ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol and CIP = ciprofloxacin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2

MIC values of plant extracts and reference antibiotics against the six bacterial strains tested in this study.

Plant or Antibiotic		MIC (µg/mL)					
		<i>S. aureus</i>	MRSA	<i>E. coli</i>	ESBL <i>E. coli</i>	<i>K. pneumoniae</i>	ESBL <i>K. pneumoniae</i>
TP	Aq	226	226	1820	1820	453	7250
	MeOH	182	182	1456	1456	1456	5825
TS	Aq	133	265	1063	1063	531	4250
	MeOH	187	187	1500	1500	1500	3000
AB	PEN	CND	>2.5	>2.5	>2.5	>2.5	>2.5
	ERY	0.625	>2.5	>2.5	>2.5	>2.5	>2.5
	TET	0.156	0.078	0.625	> 2.5	0.625	>2.5
	CHL	>2.5	>2.5	2.5	>2.5	2.5	>2.5
	CIP	0.625	0.625	CND	>2.5	CND	0.313

Noteworthy activities (400–1000 µg/mL) are indicated in blue, while good activities (100–400 µg/mL) are in green. TP = *T. phanerophlebia* fresh leaves, TS = *T. sambesiaca* fresh leaves, Aq = aqueous, MeOH = methanol, AB = antibiotics, PEN = penicillin, ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol and CIP = ciprofloxacin. CND = could not be determined as inhibition was observed at the lowest concentration tested. Values >2.5 for the antibiotics indicate a lack of growth inhibition at the highest concentration of antibiotic examined. Values highlighted in yellow indicate samples showing activity in the MIC liquid dilution assay but not in agar disc diffusion assays.

different to published data, or if their MS (mass peak, fragment peaks and ionisation ratios) were dissimilar to previously reported spectra. For all extracts, approximately 40 % of the individual mass signals were identified by comparison to the chemical and spectral databases. Only those compounds matching all reported parameters are included in Table 7.

Several noteworthy compounds were identified that may contribute to the antibacterial properties of the *T. phanerophlebia* and *T. sambesiaca* leaf extracts (Fig. 2). In particular, several tannins were putatively identified. Notably, gallic acid and the hydrolysable gallotannin corilagen were identified in both the methanolic and aqueous extracts of *T. phanerophlebia*, albeit it relatively low relative abundances (<0.1 %). In contrast, these compounds were absent, or were below the detection threshold in the *T. sambesiaca* extracts. Ellagic acid was also putatively identified in all of the *T. phanerophlebia* and *T. sambesiaca* leaf extracts, and generally in substantially higher relative abundances (generally 0.4–0.6 % relative abundance) than noted for the gallotannins.

The flavonoid naringenin was also identified in all of the extracts at noteworthy relative abundances (approximately 0.1–0.3 %). Notably, several flavonoids, including naringenin, have previously been reported to inhibit the growth of several bacteria, including *S. aureus* (Celiz et al., 2011). Furthermore, several flavonoids can also potentiate the antibacterial effects of other compounds (Ng'uni et al., 2015). Therefore, naringenin may contribute directly and/or indirectly to the antibacterial activity reported in our study. Similarly, the monoterpenoid eucalyptol (Vijayakumar et al., 2020), the chromones phomochromone B and anofinic acid (Deshmukh et al., 2015; Uzma and Chowdappa, 2021), as well as the phenolic compound quinic acid (Bai et al., 2018) have also been reported to have antibacterial activity and may therefore contribute to the growth inhibitory activity reported herein.

4. Discussion

The purpose of this study was to examine the antibacterial activities of TP and TS leaf extracts against a panel of bacterial pathogens that included *S. aureus*, *E. coli* and *K. pneumoniae*, as well as their corresponding antibiotic-resistant strains, MRSA, ESBL *E. coli*, and ESBL *K. pneumoniae*. The antibacterial evaluations were conducted using agar disc diffusion assays (as an approximation of solid phase infections) and liquid dilution assays (to approximate planktonic infections). Additionally, combinational studies were conducted to establish a quantified relationship for the plant extracts that produced MIC values in the liquid dilution assays. Qualitative phytochemical analysis was undertaken to assess the phytochemical profile of each extract in each solvent. Additionally, lethality bioassays were used as a quantitative tool to evaluate plant extract cytotoxicity and assess their safety.

Interestingly, the TP and TS leaf extracts failed to show antibacterial activity on agar against *E. coli*, ESBL *E. coli*, *K. pneumoniae* and ESBL *K. pneumoniae* bacteria. However, the liquid dilution MIC assays for *E. coli*, *K. pneumoniae* and ESBL *K. pneumoniae* bacterial strains revealed that both the aqueous and methanolic TP and TS extracts were active against these bacterial strains and produced MIC values as low as 1063 µg/mL in some cases. It is possible that both an antibacterial compound and a potentiator molecule are required and must interact in order to produce antibacterial activity. This may explain why activity was observed in liquid media, where these two molecules can freely act in partnership, while their differential rates of diffusion through agar may separate the components (Eloff, 2019), thereby not allowing extract components to potentiate the growth inhibitory activity of the growth inhibitory molecules on the semi-solid agar media. This mechanism is somewhat reminiscent of the combination formulation of amoxicillin and clavulanic acid

Table 3FIC and Σ FIC values, where relevant, for the combinations of active aqueous TP extract with antibiotics against the bacterial strains used in this study. .

Antibiotic	FIC or Σ FIC values	Bacterial species				
		<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>K. pneumoniae</i>	ESBL <i>K. pneumoniae</i>
ERY	FIC _{EXT}	1.0	-	-	-	-
	FIC _{ERY}	0.5	-	-	-	-
	Σ FIC	1.5	-	-	-	-
TET	FIC _{EXT}	0.5	0.5	0.125	1.0	-
	FIC _{TET}	0.5	0.5	0.5	0.25	-
	Σ FIC	1.0	1.0	0.625	1.25	-
CHL	FIC _{EXT}	-	-	0.5	2.0	-
	FIC _{CHL}	-	-	0.5	0.125	-
	Σ FIC	-	-	1.0	2.125	-
CIP	FIC _{EXT}	1.0	0.5	-	-	0.125
	FIC _{CIP}	0.25	0.125	-	-	4.0
	Σ FIC	1.25	0.625	-	-	4.125

Additive Σ FIC values ($>0.5 \leq 1.0$) are shown in green and antagonistic interactions (>4.0) in red. Indifferent values are $>1.0 \leq 4.0$ and are shown in blue. Synergistic (≤ 0.5) interactions were not observed. Cells containing a dash (-) indicates that combinations were not tested since the antibiotic was either inactive ($\text{MIC} > 2.5 \mu\text{g/mL}$) or it inhibited bacterial growth at the lowest concentration tested. ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol and CIP = ciprofloxacin; EXT = extract. Penicillin is not included in these assays as it did not inhibit growth of any bacteria at the highest concentrations tested, or inhibited growth at the lowest concentration tested. Similarly, ESBL *E. coli* was not included as it was not inhibited by any of the antibiotics used in this study.

(Augmentin™), whereby the clavulanic acid acts as a β -lactamase inhibitor, allowing amoxicillin to exert its antibacterial action, without being destroyed by β -lactamase (Neu and Fu, 1978).

The use of both agar and broth assays in this project has further ramifications regarding potential therapies. Bacterial growth on agar is representative of infections on epithelial surfaces such as the skin or gastrointestinal lining. Growth in broth is representative of infections occurring inside the GI or within the bloodstream. Therefore, the inhibition of *S. aureus* and MRSA growth on agar by the active TS and TP extracts indicates their potential for treating skin infections, where these bacteria are predominantly located (Del Giudice, 2020), in addition to blood-borne *S. aureus* infections (Kwiecinski and Horswill, 2020). In contrast, these extracts were only capable of inhibiting the sensitive and resistant *E. coli* and *K. pneumoniae* strains in liquid cultures. Notably, *E. coli* is a prominent cause of diarrhoea

(Levine et al., 2020) and thus resides inside the GI tract, whilst *K. pneumoniae* frequently causes urinary tract and bloodstream infections (Ali et al., 2021) and may reside in urine and blood (amongst other locations). Together, this suggests that the TP and TS extracts may be promising targets for antibacterial compound discovery, and that those compounds may effectively inhibit the growth of infectious microorganisms at multiple sites within the body. Moreover, their activities against the resistant strains (MRSA and ESBL bacteria) may provide further insights in the pursuit of therapies to combat AMR.

These findings of our study also indicate that the leaves of both *Terminalia* plant species have antibacterial activity against both gram-positive and gram-negative bacterial strains. Future experiments involving a wider panel of gram-positive and gram-negative bacterial species are warranted to determine whether the

Table 4

FIC and Σ FIC values, where relevant, for the combinations of active methanolic TP extract with antibiotics against the bacterial strains used in this study.

Antibiotic	FIC or Σ FIC values	Bacterial species				
		<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>K. pneumoniae</i>	ESBL <i>K. pneumoniae</i>
ERY	FIC _{EXT}	1.0	-	-	-	-
	FIC _{ERY}	0.5	-	-	-	-
	Σ FIC	1.5	-	-	-	-
TET	FIC _{EXT}	0.5	0.5	0.125	0.5	-
	FIC _{TET}	0.5	0.5	0.5	0.5	-
	Σ FIC	1.0	1.0	0.625	1.0	-
CHL	FIC _{EXT}	-	-	0.5	2.0	-
	FIC _{CHL}	-	-	0.5	0.25	-
	Σ FIC	-	-	1.0	2.25	-
CIP	FIC _{EXT}	1.0	1.0	-	-	0.125
	FIC _{CIP}	0.25	0.25	-	-	1.0
	Σ FIC	1.25	1.25	-	-	1.125

Additive Σ FIC values ($>0.5 \leq 1.0$) are shown in green and indifferent values ($>1.0 \leq 4.0$) are shown in blue. Synergistic (≤ 0.5) or antagonistic (>4.0) interactions were not observed. Cell containing a dash (-) indicates that combinations were not tested since the antibiotic was either inactive ($\text{MIC} > 2.5 \mu\text{g/mL}$) or it inhibited bacterial growth at the lowest concentration tested. ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol and CIP = ciprofloxacin; EXT = extract. Penicillin is not included in these assays as it did not inhibit growth of any bacteria at the highest concentrations tested, or inhibited growth at the lowest concentration tested. Similarly, ESBL *E. coli* was not included as it was not inhibited by any of the antibiotics used in this study.

antibacterial properties of these plants have a broader scope. Notably, previous work has also reported noteworthy activity for solvent extracts prepared from roots and stem bark of TS against *E. aerogenes*, *S. epidermidis*, *B. subtilis* and *M. luteus* (Fyhrquist et al., 2002) and TS and TP leaves extracted with acetone, dichloromethane and *n*-hexane against *E. coli*, *S. aureus*, *P. aeruginosa* and *E. faecalis* (Shai et al., 2008). However, the leaves of these species extracted with water or methanol has been largely overlooked. One study (Mokgoatsane, 2011) screened methanolic extracts of the TS and TP leaves against *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus* and reported strong activities. However, that study did not state the solvent used to resuspend the crude extracts, and, as such, it is difficult to compare this study with ours. Further research is required to confirm the previous results, and to extend the studies to test other species and strains. However, the efficacy of any drug still needs to be verified using *in vivo* models, as factors that influence the bioavailability of the extract components will determine the efficacy of the therapy.

While the phytochemical constituents of the *Terminalia* plant species have been extensively reviewed (Eloff et al., 2008; Cock, 2015; Zhang et al., 2019; Cock and Cheesman, 2021), limited data exists on

the compounds present in TP and TS other than a few sterol and steroid compounds (Fahmy et al., 2015). As such, metabolomics analysis of the methanolic and aqueous TP and TS leaf extracts were conducted in the present study, which identified several compounds known to possess antibacterial activity (Table 7; Fig. 2). The presence of several tannins was particularly noteworthy due the reported antibacterial activities of this class of phytochemicals (Farha et al., 2020). Gallotannins, including gallic acid, disrupt bacterial cell walls, making them more susceptible to other antibiotic compounds (Hogg and Embery, 1982; Wu-Yuan et al., 2011). Gallic acid also binds to proteins on the bacterial cell surface and inhibits several important bacterial enzymes (Wu-Yuan et al., 2011; Buzzini et al., 2008). Notably, the hydrolysable gallotannin, corilagen, was also identified in the TP extracts (but not the TS extracts). As this compound releases multiple gallic residues in mildly acidic aqueous solutions (Lu et al., 2008), it is expected that it has similar effects to gallic acid, although this remains to be verified. Ellagic acid was also identified in all of the TP and TS extracts. Several ellagitannins (including ellagic acid) also disrupt bacterial cell wall synthesis, as well as interacting with bacterial cytoplasmic membranes, with similar inhibitory affects to those of

Table 5
FIC and ΣFIC values, where relevant, for the combinations of active aqueous TS extract with antibiotics against the bacterial strains used in this study.

Antibiotic	FIC or ΣFIC values	Bacterial species				
		<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>K. pneumoniae</i>	ESBL <i>K. pneumoniae</i>
ERY	FIC _{EXT}	1.0	-	-	-	-
	FIC _{ERY}	0.25	-	-	-	-
	ΣFIC	1.25	-	-	-	-
TET	FIC _{EXT}	1.0	0.5	0.25	1.0	-
	FIC _{TET}	0.5	0.5	0.5	1.25	-
	ΣFIC	1.5	1.0	0.75	1.25	-
CHL	FIC _{EXT}	-	-	0.5	1.0	-
	FIC _{CHL}	-	-	0.5	0.062	-
	ΣFIC	-	-	1.0	1.062	-
CIP	FIC _{EXT}	1.0	1.0	-	-	0.25
	FIC _{CIP}	0.125	0.25	-	-	4.0
	ΣFIC	1.125	1.25	-	-	4.25

Additive ΣFIC values (>0.5-≤1.0) are shown in green and antagonistic interactions (>4.0) in red. Indifferent values are >1.0-≤4.0 and are shown in blue. Synergistic (≤0.5) interactions were not observed. Cell containing a dash (-) indicates that combinations were not tested since the antibiotic was either inactive (MIC > 2.5µg/mL) or it inhibited bacterial growth at the lowest concentration tested. ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol and CIP = ciprofloxacin; EXT = extract. Penicillin is not included in these assays as it did not inhibit growth of any bacteria at the highest concentrations tested, or inhibited growth at the lowest concentration tested. Similarly, ESBL *E. coli* was not included as it was not inhibited by any of the antibiotics used in this study.

the gallotannins (Buzzini et al., 2008). Notably, whilst the tannins identified in the *Terminalia* spp. extracts examined in our study have been reported to have antibacterial activities in their pure form, it remains to be determined whether they display the same effects in complex extracts. Additionally, substantially more work is required to evaluate the antibacterial and potentiating mechanisms of these compounds. We should also note that our previous work (Cheesman et al., 2023) revealed a lack of activity for ellagic acid against the bacterial species tested in the present study, despite several other previous reports indicating that the pure compound can inhibit *S. aureus* (Tavares et al., 2018) and *K. pneumoniae* (Ghudhaib et al., 2010). The roles of ellagic acid as an antibacterial agent in pure form or within plant extracts thus require further investigation.

The flavonoid naringenin (Fig. 2) was also identified in the methanolic and aqueous TP and TS leaf extracts. Several antibacterial mechanisms have previously been reported for flavonoids, including decreasing intracellular antibiotic concentrations via affecting changes in membrane structure and function (Xie et al., 2015; Cuchnie and Lamb, 2005; Górniak et al., 2019; Biharee et al., 2020).

Alterations in the cytoplasmic membrane structure may increase the fluidity of the membrane, allowing more antibiotic the pass into the cytoplasm, thereby increasing intracellular antibiotic concentrations. Alternatively, the altered membrane structure may also affect the ability of intramembrane efflux pumps to expel antibiotic molecules from the cell, also increasing their intracellular concentration. Therefore, flavonoids may also reverse some bacterial antibiotic resistance mechanisms, thereby improving the antibiotic efficacy (Górniak et al., 2019; Biharee et al., 2020). Several flavonoids have also been reported to suppress nucleic acid synthesis and alter bacterial energy metabolism, both of which also have cytostatic and cytotoxic effects (Xie et al., 2015; Cuchnie and Lamb, 2005; Górniak et al., 2019; Biharee et al., 2020). Furthermore, flavonoids also reduce bacterial biofilm formation and adhesion (Górniak et al., 2019; Biharee et al., 2020). As planktonic cells are generally more susceptible to antibiotics than biofilm associated cells, this also increases the susceptibility of the bacteria to antibiotic therapies (Xie et al., 2015; Górniak et al., 2019; Biharee et al., 2020). Future studies are required to determine whether the flavonoid components of the *Terminalia* spp. extracts have similar effects in the extracts.

Table 6

FIC and Σ FIC values, where relevant, for the combinations of active methanolic TS extract with antibiotics against the bacterial strains used in this study.

Antibiotic	FIC or Σ FIC values	Bacterial species				
		<i>S. aureus</i>	MRSA	<i>E. coli</i>	<i>K. pneumoniae</i>	ESBL <i>K. pneumoniae</i>
ERY	FIC _{EXT}	1.0	-	-	-	-
	FIC _{ERY}	0.5	-	-	-	-
	Σ FIC	1.5	-	-	-	-
TET	FIC _{EXT}	0.5	0.5	0.125	0.5	-
	FIC _{TET}	0.5	0.5	0.5	0.5	-
	Σ FIC	1.0	1.0	0.625	1.0	-
CHL	FIC _{EXT}	-	-	0.25	1.0	-
	FIC _{CHL}	-	-	0.5	0.25	-
	Σ FIC	-	-	0.75	1.25	-
CIP	FIC _{EXT}	1.0	1.0	-	-	0.5
	FIC _{CIP}	0.25	0.25	-	-	4.0
	Σ FIC	1.25	1.25	-	-	4.5

Additive Σ FIC values ($>0.5 \leq 1.0$) are shown in green and antagonistic interactions (>4.0) in red. Indifferent values are $>1.0 \leq 4.0$ and are shown in blue. Synergistic (≤ 0.5) interactions were not observed. Cell containing a dash (-) indicates that combinations were not tested since the antibiotic was either inactive (MIC $> 2.5 \mu\text{g/mL}$) or it inhibited bacterial growth at the lowest concentration tested. ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol and CIP = ciprofloxacin; EXT = extract. Penicillin is not included in these assays as it did not inhibit growth of any bacteria at the highest concentrations tested, or inhibited growth at the lowest concentration tested. Similarly, ESBL *E. coli* was not included as it was not inhibited by any of the antibiotics used in this study.

In addition to tannins and flavonoids, several other compounds that may contribute to the antibacterial activity of these extracts were also identified in TP and TS leaf extracts. The monoterpenoid eucalyptol (also referred to as 1,8-cineole; Fig. 2), modulates the fluidity of bacterial membranes, directly killing bacterial, with MIC values $<1 \text{ mg/mL}$ against many bacteria (Moghimi et al., 2017). The bacterial membrane alterations may also inhibit efflux pumps, thereby increasing intracellular concentrations of other compounds, potentiating their antibacterial effects (Mączka et al., 2021; Dmour et al., 2022; Saviuc et al., 2016). Additionally, the chromones phomochromone B (Xu et al., 2021) and anofinic acid (Elkhouly et al., 2023) kill planktonic bacteria and inhibit biofilm production. Similarly, quinic acid induces membrane damage in *S. aureus*, resulting in bacterial cell death (Bai et al., 2018). It is likely that all of these compounds identified in the TP and TS leaf extracts contribute to the antibacterial activity reported herein. However, approximately 60 % of the mass signals detected in our study were unable to be identified by comparison with chemical databases. It is possible that some of these compounds may also contribute to the antibacterial activity of these extracts and further studies are required to identify those compounds.

Combinational studies were conducted on the active TP and TS extracts to define the nature of the interactions between the extract components and the reference antibiotics. No synergistic interactions were observed, although when the extracts were mixed with tetracycline or chloramphenicol, additive responses occurred against several bacteria. This suggests that TS and TP enhance the activity of these antibiotics, although the mechanism(s) through which this occurs is yet to be determined. Both antibiotics are protein synthesis inhibitors (Wilson, 2009) but act on different ribosomal subunits, so it is likely that the antibacterial mechanisms of the antibiotics and the extracts do not overlap. In some cases, the effects between extract and antibiotic were non-interactive, so increases in antibacterial activities were not observed for those combinations. This suggests that while there would be no therapeutic benefits for using these combinations compared to using either the extract or antibiotics alone, their combined use will not reduce the antibacterial activity. In contrast, one combination induced antagonism of antibacterial activity against ESBL *K. pneumoniae* when aqueous TP, as well as aqueous or methanolic TS extracts were mixed with ciprofloxacin. This is an intriguing finding, as ciprofloxacin is generally a potent antimicrobial agent, meaning that its suppression is a significant outcome. This antibiotic inhibits

Table 7

LC–MS putative identification and % relative abundance of phytochemicals identified in the methanol (M) and water (W) plant extracts using negative ionisation mode.

RT (min)	MW (Da)	Empirical formula	Putative identification	% Relative abundance			
				TP		TS	
				M	W	M	W
0.312	310.3	C ₁₅ H ₁₈ O ₇	Orsellide C	0.11	0.13	0.07	0.16
0.312	192.17	C ₇ H ₁₂ O ₆	D(-)-Quinic acid	0.11	0.25	0.1	2
0.328	126.111	C ₆ H ₆ O ₃	5-Hydroxymethylfurfural	0.04	0.08	-	0.11
0.452	160.12	C ₆ H ₈ O ₅	α -Ketoadipic acid	-	0.01	-	-
0.544	170.12	C ₇ H ₆ O ₅	Gallic acid	0.06	0.04	-	-
7.415	636.46	C ₂₇ H ₂₄ O ₁₈	Corilagin	0.01	0.06	-	-
8.465	414.246	C ₁₈ H ₃₈ O ₁₀	Nonaethylene glycol	1.68	-	-	-
8.543	302.197	C ₁₄ H ₆ O ₈	Ellagic acid	0.42	0.6	0.61	0.35
9.547	231.183	C ₁₂ H ₂₅ NO ₃	Methyl 11-amino-10-hydroxyundecanoate	1.35	-	-	-
9.706	188.105	C ₉ H ₁₆ O ₄	Azelaic acid	1.6	3.73	3.99	1.01
9.869	217.204	C ₁₂ H ₂₇ NO ₂	(2S,3R)-2-Amino-1,3-dodecanediol	4.46	4.43	-	2.78
10.006	252.136	C ₁₄ H ₂₀ O ₄	(8aR,12S,12aR)-12-Hydroxy-4-methyl-4,5,6,7,8,8a,12,12a-octahydro-2H-3-benzoxecine-2,9(1H)-dione	-	2.04	-	1.29
10.184	233.199	C ₁₂ H ₂₇ NO ₃	2-(Hydroxymethyl)-2-(octylamino)-1,3-propanediol	-	0.84	1.1	-
10.821	344.22	C ₁₈ H ₃₂ O ₆	Gallicynoic acid F	-	0.04	-	-
11.248	272.257	C ₁₅ H ₁₂ O ₅	Naringenin	0.12	0.24	0.31	0.07
12.508	257.41	C ₁₅ H ₃₁ NO ₂	(+)-Clavaminol	4.22	-	5.49	0.01
11.684	154.249	C ₁₀ H ₁₈ O	Eucalyptol	0.01	-	-	-
11.92	328.444	C ₁₈ H ₃₂ O ₅	Corchorifatty acid	0.07	0.04	1.73	0.55
11.943	245.4	C ₁₄ H ₃₁ NO ₂	1-[(2-Hydroxyethyl)amino]-2-dodecanol	11.89	0.85	16.43	8.39
12.227	261.23	C ₁₄ H ₃₁ NO ₃	Tetradecaphytosphingosine	3.32	4.02	1.18	3.18
12.402	215.188	C ₁₂ H ₂₅ NO ₂	12-Aminolauric acid	-	0.08	5.49	-
13.41	189.17	C ₁₀ H ₇ NO ₃	Kynurenic acid	1.26	0.04	-	-
13.71	184.146	C ₁₁ H ₂₀ O ₂	Speciosin H	-	0.22	-	-
16.793	341.293	C ₂₀ H ₃₉ NO ₃	N-(1,3-Dihydroxyoctadec-4-en-2-yl) acetamide	0.12	-	0.17	-
17.084	229.24	C ₁₄ H ₃₁ NO	Lauryldimethylamine oxide	10	-	0.07	6.51
17.277	317.5	C ₁₈ H ₃₉ NO ₃	2-Amino-1,3,4-octadecanetriol	2.27	2.27	0.47	1.39
17.384	294.4	C ₁₇ H ₂₆ O ₄	Pseudoanguillosporin B	0.11	0.06	2.95	3
17.567	278.43	C ₁₈ H ₃₀ O ₂	α -Eleostearic acid	5.78	1.23	2.85	0.77
17.824	213.246	C ₁₄ H ₃₁ N	1-Tetradecylamine	0.21	-	0.33	0.12
19.035	284.39	C ₁₆ H ₂₈ O ₄	Koningin	0.99	0.03	0.08	-
19.374	303.293	C ₂₁ H ₃₇ N	2,6-Dioctylpyridine	2.79	-	3.89	1.91
19.475	299.282	C ₁₈ H ₃₇ NO ₂	Palmitoyl ethanolamide	0.69	0.66	-	-
19.662	199.194	C ₁₂ H ₂₅ NO	Lauramide	1.37	1.42	0.49	1.77
20.137	234.162	C ₁₅ H ₂₂ O ₂	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	-	-	0.09	2.41
20.459	354.277	C ₂₁ H ₃₈ O ₄	1-Linoleoyl glycerol	0.63	0.59	0.87	0.29
20.718	268.24	C ₁₇ H ₃₂ O ₂	Palmitelaidic acid methyl ester	-	-	2.81	1.82
20.913	222.24	C ₁₂ H ₁₄ O ₄	Phomochromone B	2.08	2.15	2.98	-
20.925	204.222	C ₁₂ H ₁₂ O ₃	Anofinic acid	6.18	6.49	0.17	5.21
21.239	334.49	C ₂₁ H ₃₄ O ₃	Chaetomelic acid B	0.17	0.03	1.1	-
21.479	327.278	C ₁₉ H ₃₇ NO ₃	Palmitoly alanine	1.31	1.38	-	1.65
21.548	332.5	C ₁₈ H ₃₆ O ₅	Phloionolic acid	5.6	0.42	7.68	3.68
21.611	274.251	C ₁₆ H ₃₄ O ₃	6,6'-Oxybis(2,2-dimethyl-1-hexanol)	-	-	-	3.29
21.945	156.006	C ₆ H ₄ O ₅	2,5-Furandicarboxylic acid	3.1	0.02	0.17	0.09
22.238	385.5	C ₂₃ H ₃₁ NO ₄	Monasnicotinate A	-	4.58	6.06	2.94
22.407	310.287	C ₂₀ H ₃₈ O ₂	Ethyl oleate	-	0.28	0.37	0.19
Unidentified compounds				17.49	50.25	15.73	34.09

The % relative abundances values listed in this table are not quantities. They are calculated as the % of the compound peak area compared to the total area under the chromatogram.

the bacterial DNA gyrase enzyme (Hooper et al., 1987). Therefore, the phytochemicals present in the TP and TS extracts may attenuate ciprofloxacin activity by interacting with the DNA gyrase to prevent ciprofloxacin attachment. More research is required to help elucidate this antagonism, perhaps by identifying and assaying potential DNA gyrase inhibitory phytochemicals within the extracts that may be responsible for this phenomenon.

The cytotoxicity levels of all of the extracts were determined in order to characterise the toxicity profiles of the plants. The *Artemia* nauplii lethality assay findings suggested that all extracts were non-toxic. Indeed, the brine shrimp survival following the incubation was similar to the seawater negative control. Whilst this provides an

indication that the plants extracts are safe, further studies using mammalian cell cultures should be employed to provide a more thorough assessment of their toxicity profiles to provide further evidence that they are safe for human use.

5. Conclusions

This study confirmed the antibacterial activity of methanolic and aqueous TP and TS leaf extracts against *S. aureus*, *E. coli* and *K. pneumoniae*. Additionally, the extracts also inhibited the growth of MDR bacterial strains, and were particularly good inhibitors of MRSA, with similar potency as against the antibiotic-sensitive *S. aureus* strain. Furthermore,

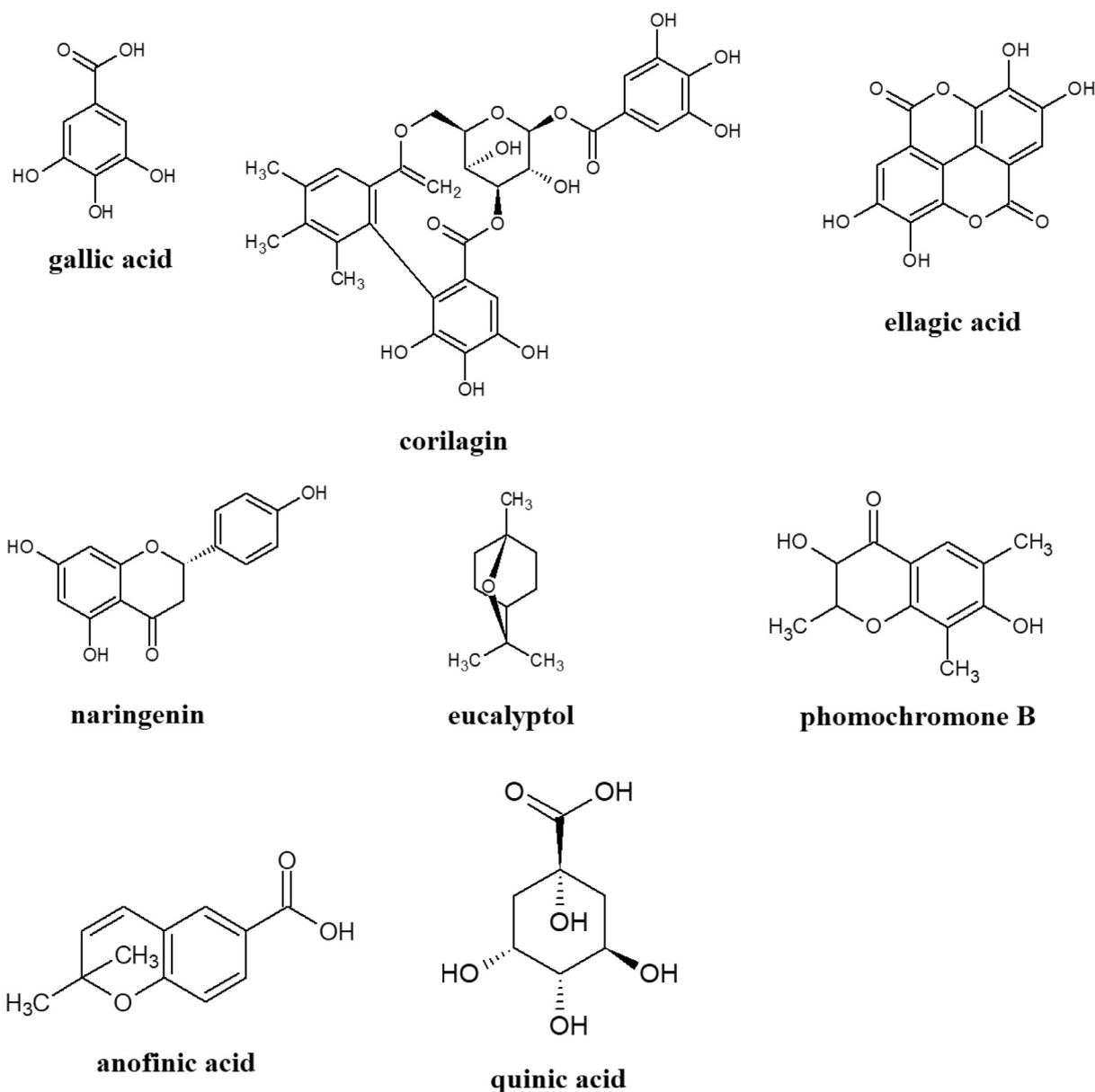


Fig. 2. Noteworthy compounds identified in the *T. phanerophlebia* and *T. sambesiaca* leaf extracts, including the tannins gallic acid, corilagin, ellagic acid, the flavonoid naringenin, the monoterpene eucalyptol, the chromones phomochromone B and anofinic acid, as well as the phenolic compound quinic acid.

combinations of the extracts with some conventional antibiotics (particularly tetracycline) had substantially potentiated antibacterial effects, indicating that extract components block bacterial resistance mechanisms, perhaps by efflux pump inhibition. The lack of toxicity of the extracts and their antibacterial effects against MRSA highlight these extracts as targets for antibacterial drug development.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Chantelle Florenca: Writing – original draft, Investigation, Formal analysis. **Gagan Tiwana:** Software, Investigation, Formal analysis. **Gary D. Grant:** Supervision, Project administration. **Ian E. Cock:** Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Investigation, Formal analysis, Conceptualization. **Matthew J. Cheesman:** Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Investigation, Formal analysis, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2024.08.039.

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