scientific reports

OPEN

Metabolic profiling and antibacterial activity of secondary metabolites extracted from the endophytic bacteria of *Combretum erythrophyllum*

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Antibiotic resistance continues to pose a significant global challenge associated with increased rates of illness and death. The emergence of multidrug resistance patterns in pathogenic bacteria poses a challenge in their treatment, rendering them unmanageable with traditional antibiotics in the foreseeable future. Thus, prioritising innovative pharmaceuticals for therapy and exploring alternate approaches to combat antimicrobial resistance is of utmost importance. Endophytic bacteria derived from medicinal plants are reservoirs of active therapeutic compounds, offering a promising alternative for discovering novel and therapeutically bioactive compounds. This work involved the isolation and identification of four endophytic bacteria from *Combretum erythrophyllum*. The antibacterial activity and metabolic profiling of the secondary metabolite extract were assessed via the microdilution technique and gas chromatography data analysis, respectively. The assessed extract showed wide-ranging effectiveness against Gram-positive and Gram-negative bacteria, with a particular preference for *Klebsiella aerogenes* (ATCC 27853) at a minimum inhibitory concentration of 125 µg/mL. The analysed secondary metabolites revealed phytochemicals, namely terpenoids, ketones, phytosterols, phenols, alkanes, and fatty acid methyl esters (FAME) as the bioactive constituents. In conclusion, this study underscores the importance of the endophytic bacteria of *C. erythrophyllum* as a potential source of active natural bioactive compounds for antibiotic therapy.

Keywords Antibiotics, *Combretum erythrophyllum*, Endophytes, *Klebsiella aerogenes*, Secondary metabolites

Antibiotic resistance (AR) has become an ongoing global concern that has led to a high mortality rate and economic burden in the 21st Century[1]. Infectious or pathogenic bacteria in humans could develop resistance to the antibiotics repeatedly used to combat them[2] .Thus leading to fewer antibiotic options for treatment. The global impact of antimicrobial resistance (AMR) has led to substantial morbidity, and projections suggest that the associated mortality could reach up to 10 million by the year 2050. Furthermore, this phenomenon is anticipated to result in a collective economic loss of USD 100 trillion on a global scale[3,4]. A significant cause of AR in bacteria is mostly due to the selective pressure of the misuse of antibiotics. AR can also be caused by the horizontal transmission of resistance genes via acquisition, expression or selection. The global overuse of antibiotics has enhanced the widespread bacterial resistance with the likelihood of having a 'post-antibiotic age' in which there would be few or no antibiotics for treating infectious diseases[5]. As a result of the possibly dreadful implications for human health posed by AR and the limited number of antibiotics developed to combat this, the search for safer antibiotics continues.

Endophytes refer to microorganisms, predominantly fungi or bacteria, that inhabit regions of plant tissues, including the leaf, root, stem, and seed, without eliciting any detrimental effects on the host plant[6]. The term "endophyte" refers to microorganisms that reside within the tissues of plants. These microorganisms are frequently observed in the phyllosphere (the aerial parts of plants) and the rhizosphere (the soil surrounding plant roots)[7]. Endophytic microorganisms are isolated from the internal tissues of plants after serialised surface sterilisation of the host plant tissue. Endophytic microbes stimulate plant growth through the production of

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phytohormones, which aid the improvement of nutrition via bi-directional nutrient transfer and also improve plant health by protecting them against pathogens[6,8,9]. The symbiotic relationship between the plant and the endophytes protects the plants against harmful environmental conditions due to the production of bioactive metabolite and biotechnologically significant enzymes[10,11]. Furthermore, endophytes are known to protect against pathogens and parasites while increasing the host plant's resistance to drought and low soil fertility. As a result of their ability to produce or possess similar metabolites to their host plant, they often shield the plant from abiotic and biotic stress[12].

Consequently, endophytes have gained pharmaceutical and industrial relevance due to their capability to secrete secondary metabolites which can be used as natural antioxidants, antimicrobial agents (antibacterial, antifungal and antiviral), bio-control agents, anticancer agents, antidiabetic agents, immunosuppressant, and pesticide[13–15]. Secondary metabolites are plant specifics that are generated as part of the plant's defense system against invading pests and pathogens. In a previous study, the secondary metabolites of the endophyte of *Combretum molle* were shown to have an antibiotic effect against *Bacillus cereus* and *Staphylococcus aureus*[16]. Extracted secondary metabolites of the endophytic *Bacillus velezensis* Bvel1 served as a biocontrol agent against the causative agent of bunch rot in the post-harvesting of grapes[17].The identification of these metabolites can be done by different techniques, such as gas chromatography coupled with mass spectrometry (GC–MS), which has the inherent capability to detect volatile and semi-volatile compounds while distinguishing them by their masses. GC–MS operates with a constant electron ionization energy which is able to produce reproducible fragmentation pattern, efficient sensitivity and fewer matrix effects that encourages its regular use for the analysis of metabolites[18].

Combretum erythrophyllum (CE) is an indigenous deciduous arboreal species found predominantly in the southern region of Africa, particularly in South Africa. CE is a plant species that exhibits autonomous growth and frequently serves the dual function of providing shade and enhancing aesthetic appeal as an ornamental plant. This particular flora species is known by various appellations, including River bushwillow in English, Umdubu-wehlandze in Zulu, Muvuvhu in Venda, Modibo in Northern Sotho, and Riviervaderlandswilg in Afrikaans[19]. The utilisation of extracts derived from CE leaves, seeds, and barks mainly focuses on therapeutic applications owing to their potent antibacterial properties. Prior scientific research has demonstrated that the leaf and extract of this particular specimen possess a composition of flavonoids (4′,5-dihydroxy-7-methoxyflavonol [rhamnocitrin] and 4′,5,7-trihydroxyflavonol [kaempferol])[20], alkaloids (quinine and strychnine)[21], phenols (Combretastatin)[22] and essential oils, all of which exhibit notable antibacterial properties[23,24].

This study aimed to isolate and identify the microbial symbiotic endophytic bacteria from C. *erythrophyllum* in order to explore the bioactive usefulness of endophytes. In addition, the objective is to conduct metabolic profiling and assess the antibacterial efficacy of the secondary metabolites derived from the isolated endophytic bacteria against certain pathogenic bacteria strains.

Materials and methods

Isolation of endophytic bacteria

The process utilised to isolate the endophytes from the plant (including its leaves, seed, and stem) was as described by Jasim et al.[25]. In brief, a portion of the plant was meticulously rinsed with tap water to remove any grime or filth. Clean samples of the plant were subsequently divided into smaller segments (approximately 2 cm in length). After soaking the sterile distilled water (SDW) treated plant in Tween 80 for approximately ten minutes while vigorously shaking, the plant was subsequently cleansed. The plant parts were treated for 10 min with 1% sodium hypochlorite (NaOCl) after being immersed in 70% ethanol for one minute. In addition, the plant specimens underwent five serial washes using SDW. The final wash was applied to nutrient agar (NA) dishes as a control.

To extract the bacterial endophytes, the sterile internodes of the plants were severed. With phosphate-buffered saline (PBS), the residual plant components were macerated. The serial dilution (10^{-3}) was applied in 0.1 mL portions onto NA plates. The plates were incubated at 37 °C for twenty-four hours, and the bacterial colony growth was observed. Pure bacterial colonies were obtained by re-cultivating the observed colonies on sterile NA plates. For subsequent use, the bacterial isolates were preserved in glycerol stocks (50% glycerol) and frozen at −80 °C.

Morphological identification of bacteria by gram staining

As Collins et al.[26] demonstrated, a pure colony of each endophytic bacteria was subjected to the Gram staining procedure to ascertain morphological attributes such as morphology and Gram stain reaction. 100x magnification compound bright-field microscopy (OLYMPUS CH20BIMF200) was utilised to examine the Gram-stained specimens.

Morphological characterization of bacteria by scanning electron microscope

Bacterial isolates were incubated for one day (24 h) at 37℃. The colonies' samples were obtained and subsequently subjected to Scanning Electron Microscope (SEM) analysis in accordance with the methodology adapted from Mamonokane et al. 2018 [16]. To summarise, the specimens underwent a 10-minute centrifugation at 1100x g, after which the supernatants were discarded. The granules were then fixed for one hour at room temperature in a solution of 2% glutaraldehyde and 1% formaldehyde (1:1 v/v). Following this, the specimens underwent a 10-minute centrifugation at 1100x g, during which the supernatant was discarded. The samples were subjected to serial dehydration using 30, 50, 70, 90, 95, and 100% ethanol concentrations for intervals of 10 min. Samples were stored at 4℃ overnight. The dehydrated specimens were affixed to SEM substrates and gold-coated utilizing an Emscope SC 500. They were subsequently examined through a Tescan VEGA SEM (VEGA 3 LHM, AVG9731276ZA) linked to a monitor operating at 10 kV.

Molecular identification of the bacterial endophytes using the 16 S rRNA

Extraction of genomic DNA

Each bacterial isolate was injected into nutrient broth (NB) after growing a mature colony on nutrient agar (NA). The cultures were incubated overnight at a temperature of 37°C. The cultures underwent centrifugation at a force of 1300 x g for a duration of 5 min, following which the liquid portion above the sedimented material was removed and discarded. The DNA extraction procedure involved the utilisation of a ZR fungal or Bacterial DNA kit (Zymo Research, catalogue No R2014) in accordance with the instructions provided by the manufacturer. The quantification of the extracted DNA was performed using the NanoDrop ND-2000 UV-Vis spectrophotometer, manufactured by Thermo Fisher Scientific in the United States.

Polymerase chain reaction amplification and sequencing

The amplification of the 16 S rRNA gene for each bacterial isolate was conducted using a polymerase chain reaction (PCR) method, following the technique and utilizing the primers as described by Yeates et al.[27]. The PCR products underwent purification using ExoSAP-it™ in accordance with the manufacturer's guidelines and were subjected to sequencing at Inqaba Biotechnical Industries (Pty) Ltd, located in Pretoria, South Africa.

Phylogenetic analysis

The sequences acquired were subjected to a screening process for the detection of chimeras, utilizing the DECIPHER tool[28]. Using the Basic Local Alignment Search Tool (BLAST), the screened sequence of each endophyte was compared to the nucleotide collection database of the National Centre for Biotechnology Information (NCBI) in order to identify bacterial species that are closely related and have high percentages of similarity. Following the BLAST search, all phylogenetic analyses were conducted using MEGA 7[29]. The alignment between the isolates of this study and the species obtained from the BLAST search was performed utilizing the Multiple Sequence Comparison by Log-Expectation (MUSCLE) method[30]. With 1000 replications, maximum likelihood trees of the obtained homologous sequences were deduced utilizing Using the Jukes-Cantor Model[31]. Additionally, 1000 replicates of bootstrap analysis were used to regulate branch support. The GenBank (https://www.ncbi.nlm.nih.gov/genbank) has been updated with the 16 S rRNA gene sequences of the bacterial isolates which were investigated in this study. Based on BLAST homology percentages and phylogenetic outcomes, the names of endophytic bacterial isolates were determined.

Extraction of secondary metabolites from endophytic bacteria

Four 2 L (L) Erlenmeyer flasks were utilised to prepare individual volumes of one litre of Nutrient broth (NB). Subsequently, the flasks were subjected to autoclaving at a temperature of 121 °C for a duration of 15 min. Each 2 L flask, which contained NB and had a volume of 2 L, was inoculated with a separate bacterial isolate. The flasks were then placed in a shaking incubator and incubated at a speed of 150 rpm for a period of 7–10 days at a temperature of 37℃[32]. Following the designated incubation period, the culture underwent centrifugation at a speed of 10,000 revolutions per minute for a duration of 30 min in order to eliminate the biomass. The extraction of secondary metabolites involved the addition of equal quantities of ethyl acetate and chloroform (1: 1 v/v) to the supernatant in a separating funnel, followed by vigorous shaking for a duration of 2 min. The organic solvent layer was gathered within a conical flask. Subsequently, the organic layer was subjected to concentration using a vacuum rotary evaporator operating at a temperature of 55 °C. Subsequently, the crude secondary metabolite extract was transferred to a sterile vial and allowed to undergo evaporation at ambient temperature.

Metabolic profiling of CE extract

Sample Preparation for metabolic profiling

The dried secondary metabolite extract of CE was reconstituted using 1 mL of methanol of chromatographic grade. Subsequently, the substance was subjected to filtration and subsequently transferred into vials of a dark amber colour for the purpose of analysis.

GC-HRTOF-MS analysis

The analysis was done using a previously described method by Adebo et al. 2019,[33]. The method is fully described in S.1. Further multivariate data analysis was carried out by loading the processed data into the SIMCA 18.0.1 software version (Umetrics, Umea, Sweden).

Antibacterial activity of the secondary metabolites of CE endophytic bacteria

The microdilution technique was used in the evaluation of the minimum inhibitory concentration according to a previously described method by Fanoro et al. 2021[34]. The supplementary material (S2) has a detailed description of the process.

Results

Morphological and molecular identification of the CE endophytic bacteria

Endophytes were isolated from different parts of the medicinal plant *Combretum erythrophyllum* at the fruiting stage. 16 S rRNA sequencing technique was used to identify the isolated bacterial endophytes. In total, four bacterial isolates were isolated from the plant, comprising two from the seed (Org A and Org F) and one each from the stem (Org F) and leaf (Org PVO) [Table 1]. A scanning electron microscope was also used to determine the shapes of the isolated endophytes (Fig. 1A-D).

No	Bacteria designation	Plant part	GENBANK ascension no	Assigned bacteria name	Gram staining	Shape
1	Org A	Seed	MG009455	*Ralstonia* sp.	Gram-negative	Rod
2	Org C	Stem	MG009454	*Staphylococcus* sp.	Gram-positive	Cocci
3	Org F	Seed	MG009456	*Methylobacterium radiotolerans*	Gram- negative	Rod
4	Org PVO	Leaves	MG009453	*Pantoea vagans*	Gram- negative	Rod

Table 1. Bacterial isolates from *Combretum erythrophyllum*.

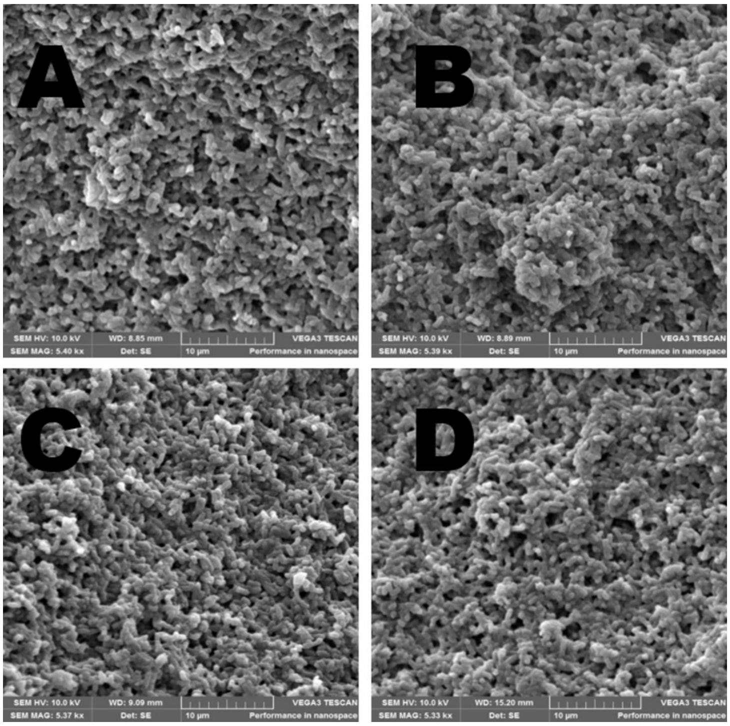

Fig. 1. Scanning electron micrograph showing the cell morphology of the four isolated endophytic bacteria (**A**) *Raslsonia* sp. [Org A] (**B**) *Staphylococcus sp.* [Org F) (**C**) *Methylobacterium radiotolerans* [Org C] (**D**) *Proteus vulgaris* [Org PVO].

Phylogenetic relationship

Figure 2 shows the maximum likelihood phylogenetic tree that shows the phylogenetic relationship of the isolated endophytes to their closest related bacterial species. The BLAST search showed that the listed bacterial isolates in Table 1 shared 99% homology to the species of bacteria belonging to the genus *Ralstoni*a, *Staphylococcus*, *Methylobacterium* and *Pantoea* as indicated in Fig. 2. Based on the above, the following names were assigned to the isolated endophytes *Ralstonia* sp., *Staphylococcus* sp., *Methylobacterium radiotolerans*, and *Pantoea vagans*.

Overview and exploration of the acquired GC-MS data

The utilisation of the high-resolution GC-TOF-MS platform allowed for the simultaneous detection of numerous analytes with a high level of sensitivity, thereby facilitating a comprehensive characterisation of the metabolic profile of both the CE extract and its endophytic bacteria. This capability stems from the inherent chemo-diversity and multi-dimensionality of the extracted metabolome. Table S1-S5 summarises the obtained metabolites, retention time (R_t), metabolite class and their biological activity. Volatile compounds in different chemical classes were identified from the CE leaf extract and the secondary metabolite of the endophytic bacteria. Diverse metabolite classes such as phenols, esters, alcohols, ketones, terpenoids, phytosterols, alkanes, amides, fatty acid and its derivatives (fatty acid methyl esters (FAME), fatty acids amides, and fatty acid furyl esters) were found in the secondary metabolite and the water extract of the CE leaf. In order to compare the metabolites between the CE leaf extract and the four endophytic bacteria, a Venn diagram was constructed using a freely available bioinformatics tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). Figure 3 shows the constructed Venn diagram, which reveals the relationship between the metabolites of the CE leaf extract and that of the four endophytic bacteria. Principal component analysis (PCA) was used to study the metabolite profile of the secondary metabolites of the isolated endophytes and the CE leaf extract. The PCA score and the loading scatter plot are shown in Fig. 4a and b respectively. Also, a pie chart was used to group the metabolites into different classes of compounds (Fig. 5).

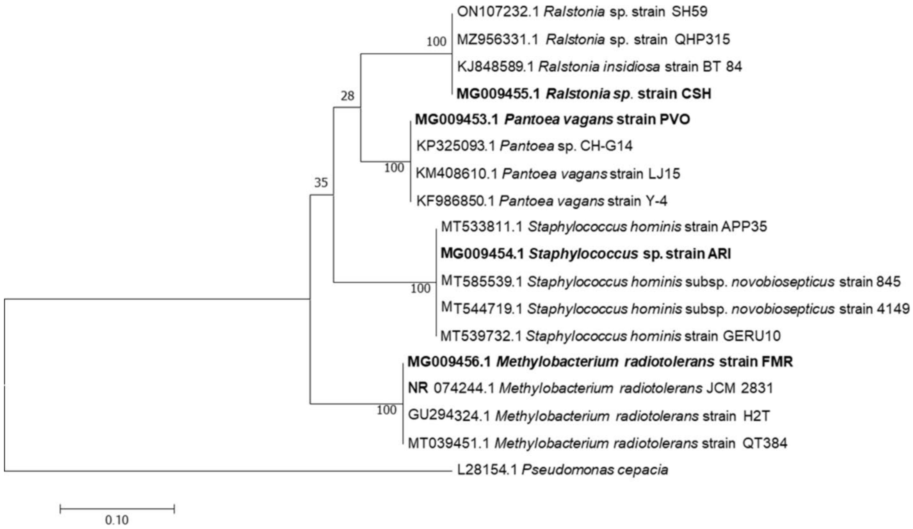

Fig. 2. Maximum likelihood phylogenetic tree of *Ralstonia* sp. (MG009455.1), *Staphylococcus* sp. (MG009454.1), *Methylobacterium radiotolerans* (MG009456.1), Pantoea *vagans* strain (MG009453.1) and their closest neighbour based on the 16s RNA gene sequences obtained from the NCBI BLAST search. *Pseudomonas cepacian* was included as an out-group. The bootstrap values are indicated on the nodes.

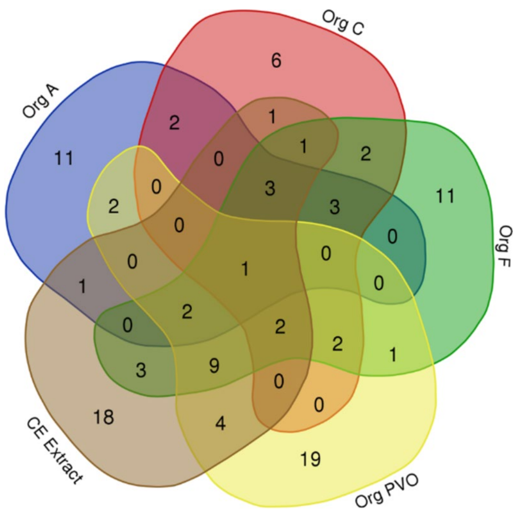

Fig. 3. Venn diagram showing the relationship among the CE leaf extract and metabolites of the four isolated endophytic bacteria.

Result of the antibacterial study of the secondary metabolites of CE endophytic bacteria

The minimum inhibitory concentration approach was employed to assess the antibacterial activity of the secondary metabolites derived from the four endophytic bacteria. The secondary metabolite extracts derived from Org A exhibited an inhibitory effect on all of the pathogenic microorganisms that were subjected to testing. The minimum inhibitory concentration (MIC) values of the secondary metabolite extracts were found to be high (ranging from 4000 to 125 µg/mL) for all of the pathogenic organisms that were subjected to testing, except for *Klebsiella aerogenes*, which exhibited a very low MIC value of 125 µg/mL Similarly, secondary metabolite extracts derived from Org C and Org PVO showed an inhibitory effect against all the pathogenic organisms that were examined. The secondary metabolite extracts derived from Org F exhibited inhibitory properties against certain species; however, no inhibitory effects were identified against *Escherichia coli* and *Proteus vulgaris*, *Bacillus cereus* and *Klebsiella pneumonia* (Table 2).

Discussion

The process of surface sterilisation of plant material holds great importance in the research of endophytic bacteria, particularly when considering the objective of obtaining uncontaminated isolates[16]. To isolate endophytic bacteria, the leaves, seeds, and stems of C. *erythrophyllum* were subjected to surface sterilisation. The

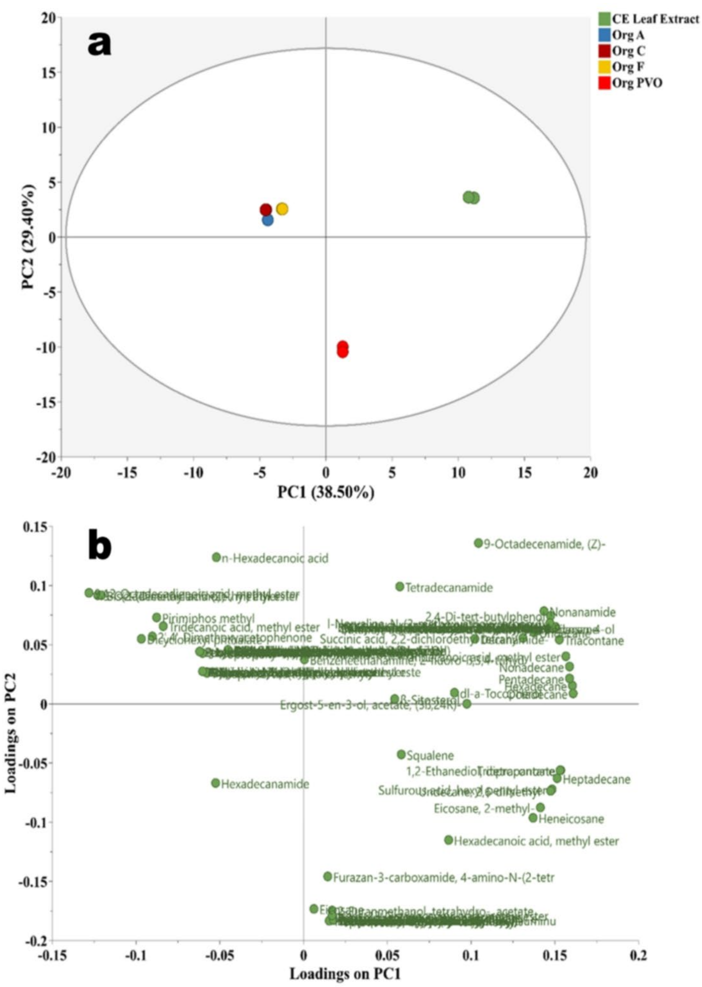

Fig. 4. Principal component analysis (PCA) score (**a**) and loading scatter plots (**b**) obtained from identified metabolite compounds of Org A, Org C, Org PVO, and CE Leaf extract.

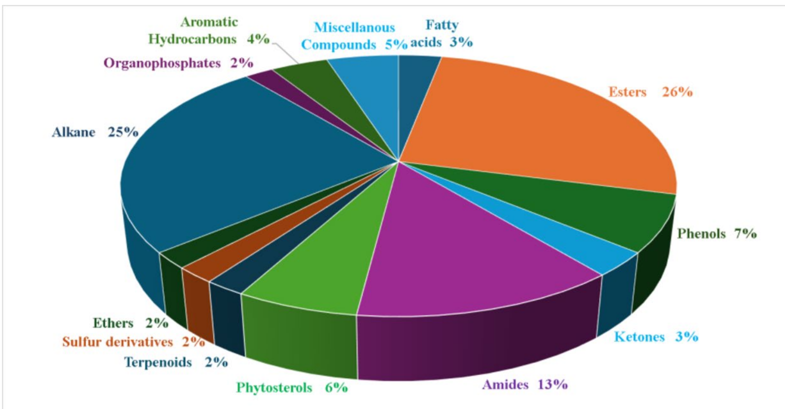

Fig. 5. Pie chart displaying the percentage distribution of the metabolite groups in the CE endophytes and leaf extract.

surface sterilisation process yielded no growth on the control plate, indicating a satisfactory process. Therefore, the bacterial colonies that were isolated can be considered authentic endophytes. The primary emphasis of the bacterial identification process revolved around the assessment of their Gram reaction and appearance. The Gram stain reaction results indicated that one (Org C) of the identified bacterial endophyte isolate was Gram-positive (Gram + ve) while the other three (Org A, Org F and Org PVO) isolates were Gram-negative (Gram −ve). The SEM micrographs shown in Fig. 1A-D further confirm the result of the morphology of the shapes obtained from the Gram staining reaction (Table 1). The uniformity of the cells, as seen in the SEM micrographs, indicates the purity of the bacterial cultures. Furthermore, Table 1 shows the location of the CE plant where endophytic bacteria were isolated from. Having two organisms isolated from the seed may be attributed to the abundance of the phyllosphere present in the seed[8].

The outcome of the BLAST analysis conducted on the 16 S rRNA gene sequences revealed the presence of distinct bacterial taxa. The identification of the isolated endophytic bacteria was conducted by comparing their genetic sequences to those of other strains in the GenBank database, resulting in a 99% similarity match. Each identified endophytic bacterium was then assigned a unique GenBank accession number, which serves as a distinct identifier within the NCBI database. These accession numbers are based on the 16 S rRNA submissions associated with each endophyte. The NCBI BLAST analysis of the 16 S rRNA gene sequences yielded four distinct outcomes of bacterial genera, which show the microbial diversity found in *Combretum erythrophyllum.* The diversity of endophytes is usually based on the sampling season, tissue type, plant age, and the environment or geographical location[25]. This could possibly have accounted for the different genera isolated from the CE plant at the time of sampling. Therefore, it is plausible that C. *erythrophyllum* could be associated with various or diverse genera of endophytic bacteria. The phylogenetic analysis showed that the isolated bacteria endophytes align with their closest related bacterial species, which are *Ralstonia sp.*, *Staphylococcus sp.*, *Methylobacterium radiotolerans*, and *Proteus vulgaris*.

High-resolution GC-TOF-MS could be used to detect different analytes of the metabolites of the endophyte and plant due to their inherent chemo-diversity and multi-dimensionality of the extracted metabolome. In the metabolomics analysis, only one metabolite, which was a phytosterol, named ß-Sitosterol was the only common metabolite among the CE leaf extract and the four endophytic bacteria. Each of the samples had unique compounds or metabolites that were not found in the other. The outward part of the Venn diagram with distinct colours shows the metabolites that were discrete and unique, and the segments with merged colours show that the metabolites were found in two or more of the bacteria endophytes Eleven (11), six (6), eleven (11), nineteen (19) and eighteen (18) unique metabolites were Org A, Org C, Org F, Org PVO and the CE extract, respectively. This signifies that although their origin was from the same plant, they possess unique bioactive compounds. The highest similarity between the CE Leaf extract and endophytic bacteria was found in the secondary metabolite extracts of Org PVO, which was isolated from the leaf. This confirms the protective role of endophytes in their symbiotic relationship. with plants (Fig. 3). Bacterial endophytes possess an intrinsic capacity to synthesise or produce unique and novel natural compounds that exhibit dual antimicrobial properties against both bacteria and fungi[35].

PCA is able to distinguish data based on their differences and similarities. PC1 ver-sus PC2 score plots of the PCA model (Fig. 4a) showed a clear difference in the composition of the metabolites of the endophytes and the plants, with the sum of PC1 and PC2 accounting for 67.90% of the variation. The PCA shows Org A, Org C, Org F, Org PVO, and CE leaf extract with PC1 and PC2 values of 38.5% and 29.4%, respectively. The observed separation and clusters show the differences and possible similarities in metabolites based on different characteristics[12]. The resulting PCA plot in Fig. 4a shows that the secondary metabolite extracts from the isolated endophytes were grouped in plant parts from which they were isolated. PC1 separated Org PVO and the CE leaf extract to the right, showing the relatedness of Org PVO to the CE leaf extract from which it was isolated. On the other hand, Org A, Org C and Org F were separated to the left, showing their relatedness to each other. The observed groupings and clustering of the secondary metabolite extracts can be ascribed to the similarities in the investigated metabolites. The separation of the secondary metabolite extracts of Org A, Org C and Org F from that of Org PVO shows a difference in their metabolite composition, showing that metabolites of endophytes isolated from the seed and stem of CE are similar but different from that of the leaves. The PCA loading plot shown in Fig. 4b shows the visualization of metabolites, contributing to the observed differences and similarities of grouping. The distribution of the metabolite classes of the compound was analysed using a statistical tool, a pie chart. Esters and alkanes (long chain) were the most abundant metabolites found in all the analysed samples, with 26% and 25%, respectively. Amide (13%), Phenols (7%) and Phytosterols (6%) and Aromatic Hydrocarbons (4%) were found to be relatively abundant. Fatty acids, Ketones, Sulfur derivates, Ethers, Aromatic hydrocarbons and Organophosphates comprised 2–3% of the notable metabolites. The observed miscellaneous compounds were only 5%.

The production of bioactive compounds by endophytic bacteria is comparable to that of their host plant, owing to the symbiotic interaction between the two entities. Previous studies have shown the antibacterial activity of metabolites derived from Pseudomonas spp. and Enterobacter spp., which were isolated from C. *molle.* These extracts have demonstrated efficacy against bacteria belonging to both the gram-positive and gram-negative categories. Their antibacterial action was attributed to the presence of flavonoids and saponins derived from the host plant[16]. The metabolites of a plant endophyte, *Staphylococcus hominis*, isolated from jute seed, showed an antibacterial effect towards *Staphylococcus aureus* SG511 due to the effect of the homicorcin compound present in the extract. The results obtained from this study, as shown in Table 2, indicate that the secondary metabolite extracts derived from *Combretum erythrophyllum* possess the potential for utilization in the development and advancement of traditional and pharmaceutical antibiotics for antibiotic therapy.

Conclusion

Four different endophytic bacteria, specifically *Ralstonia* sp., *Staphylococcus* sp., *Methylobacterium radiotolerans*, and *Pantoea vagans*, were obtained from various parts of CE, including the seed, stem, and leaves. Secondary metabolites were extracted from the isolated endophytic bacteria. The leaf extract of CE and the extract of its isolated endophytic bacteria were subjected to metabolomics analysis using GC-HRT. The results of this analysis

No.	Bacterial strain	Secondary metabolites of Org A (µg/mL)	Secondary metabolites of Org C (µg/mL)	Secondary metabolites of Org F (µg/mL)	Secondary metabolites of Org PVO (µg/mL)
1	*Staphylococcus epidermidis* (Se) (ATCC 14990)	500	1000	4000	1000
2	*Staphylococcus aureus (Sa) (ATCC 25923)*	500	500	4000	2000
3	*Proteus mirabilis* (Pm) (ATCC 7002)	250	500	2000	500
4	*Escherichia coli* (Ec) (ATCC 25922)	1000	1000	–	2000
5	*Bacillus cereus* (Bc) *(ATCC 10876)*	1000	250	4000	1000
6	*Proteus vulgaris (Pv) (ATCC 6380)*	1000	1000	-	2000
7	*Bacillus subtilis (Bs) (ATCC 19659)*	4000	4000	-	2000
8	*Klebsiella pneumoniae (Kp) (ATCC 13822)*	4000	4000	-	2000
9	*Klebsiella oxytoca* (Ko) (ATCC 8724)	500	1000	4000	2000
10	*Klebsiella aerogenes (Ka) (ATCC 27853)*	125	1000	4000	2000
11	*Mycobacterium smegmatis* (Ms) (MC 2155)	1000	1000	4000	2000
12	*Enterococcus faecalis* (Ef) (ATCC 13047)	4000	4000	-	2000

Table 2. Results of the MIC antibacterial study of the secondary metabolites isolated from each of the endophytic bacteria.

revealed the presence of many phytochemicals, including terpenoids, ketones, phytosterols, phenols, alkanes, and fatty acid methyl esters (FAME). The secondary metabolite extracts exhibited a broad-spectrum inhibitory effect against both gram + ve and gram -ve pathogenic bacteria. The antibacterial efficacy can be ascribed to the diverse categories of bioactive compounds found in the metabolites. The findings of this study indicate that the secondary metabolites derived from C. *erythrophyllum* have the potential for utilisation in the development of novel antibiotics and other pharmaceutical products. Furthermore, the use of endophytic bacteria for prospecting pure bioactive compounds for ground-breaking drug discovery possibilities is further confirmed.

Data availability

The bacteria isolated in this study were identified using the amplification and sequencing of the 16 S rRNA gene. The resulting data has been deposited in the GenBank (https://www.ncbi.nlm.nih.gov/genbank) with ascension numbers MG009453, MG009454, MG009455 and MG009456. All other data generated or analyzed during this study are included in this published article and its supplementary information file.

Received: 18 July 2024; Accepted: 22 April 2025
Published online: 27 April 2025

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Acknowledgements
The authors would like to thank the National Research Foundation (N.R.F) under the Competitive Programme for Rated Researchers (CPRR), grant no 129290, the University of Johannesburg Research Committee (URC) and the Faculty of Science Research Committee (FRC) for the financial support. The authors would also like to thank Christopher Willis, Andrew Hankey, and Solomon Nenungwi of the Walter Sisulu National Botanical Garden, Roodepoort, for their provision of the plant.

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Olufunto T. Fanoro: Conceptualization, Investigation, Methodology, Data curation, Writing Original Draft, Software, Formal analyses, Visualization and Validation. Oluwatobi S. Oluwafemi: Supervision, Funding, Conceptualization, Validation and Review. All authors contributed to the study conception and design. O.T.F. and and O.S.O. conceived and designed research. O.T.F. prepared materials, conducted experiments, analyzed data, and wrote the manuscript. O.S.O. was awarded the funding and reviewed the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-99709-y.

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