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Chemical profiling and inhibitory effects of selected South African plants against phytopathogenic bacteria and fungi of tomato

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ABSTRACT

Soilborne pathogens are economically important, causing great losses in agricultural production globally. The high cost and toxicity of antibiotics, coupled with the development of drug-resistant bacteria, has awakened interest in finding alternative methods of plant-pathogen control. This study aimed to screen extracts of selected plants against phytopathogenic bacteria and fungi that infect tomatoes, and to profile their chemical constituents. The antimicrobial activity of acetone, water and dichloromethane: methanol (DCM/MeOH = 1:1) extracts from leaves of ten plants was examined against five phytopathogenic bacterial strains and one fungal strain using a serial microplate dilution method to determine the minimal inhibitory concentration (MIC) values. Gas chromatography-mass spectrometry (GC–MS) was used for profiling constituents in the acetone and DCM/MeOH extracts. The MIC values indicated weak antibacterial activity of all the water extracts against tested bacterial strains. Acetone and DCM/MeOH extracts of *Leucosidea sericea* and *Searsia lancea* had very good to outstanding antibacterial activity against most of the tested bacteria with MIC values ranging between 19.5 and 78 μg/mL. All extracts were not active against *Fusarium* spp. except for the acetone extract of *Cotyledon orbiculata* and the water extract of *Leonotis leonurus* which inhibited the growth of *F. oxysporum* with MIC = 39 and 97.5 μg/mL after 24 h, with further incubation resulting in MICs of 156 and 469 μg/mL respectively. The GC–MS analysis of the acetone and DCM/MeOH extracts indicated the major peaks of 9-octadecenamide, (Z)-, octadecanoic acid and dodecanamide which were present in almost all the extracts. The 9-octadecenamide was found to be the most highly concentrated compound in most extracts. *Leucosidea sericea* and *S. lancea*, therefore, contain bioactive compounds that may be used as broad-spectrum antimicrobials against phytopathogenic bacteria and fungi.

1. Introduction

Tomato is ranked as the first vegetable with respect to world vegetable production and accounts for 14 % of world vegetable production (US$ 1.6 billion market value) (FAO, 2010). In South Africa, it is the second most important cultivated vegetable after potato (FAO, 2016), and the Department of Agriculture, Forestry and Fisheries as it was then known postulated that tomato alone constitutes close to 17 % of the total gross value of vegetable production in 2018 (DAFF, 2019). Tomatoes are produced in all South African provinces, with Limpopo province being the major production area both in the Northern Lowveld and far northern areas. The other main producing areas are the Onderberg area of Mpumalanga province and Border area of the Eastern Cape province (DAFF, 2019). The tomato crop yield is primarily limited by a variety of causes, including biotic constraints. Diseases caused by soilborne pathogens affect both production and development of crops. Seedling, root rot and vascular diseases are amongst the important diseases caused by soilborne pathogens (Katan, 2017). According to Kumar and Prasad (2020), post-harvest losses due to fungal and bacterial infections are estimated to be 25 % worldwide. The high yield loss is a major concern to producers and threatens food security globally (Savary et al., 2012).

Bacterial diseases caused by *Clavibacter, Ralstonia* and *Xanthomonas* species are a major concern in the production of tomatoes and other crops. *Clavibacter michiganensis* subsp. *michiganensis* was reported by Wallis (1977) cited in Jahr et al. (1999) as the causal agent of bacterial canker and wilt in tomato which occurs through wounds, followed by the invasion of the xylem vessels, which establishes a systemic vascular disease. It is a seed-borne phytopathogenic bacterial species that causes substantial economic losses (Dutta et al., 2014). The *Ralstonia solanacearum* species complex is comprised of three species: *R. solanacearum, R. pseudosolanacearum* and *R. syzygii*

Abbreviations: DCM/MeOH, dichloromethane methanol; GC-MS, gas chromatography-mass spectrometry; MIC, minimal inhibitory concentration

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(Prior et al., 2016). *Ralstonia solanacearum* has a wide host range and infects crops in the vascular stems, causing a brown discoloration and drops of white or yellowish bacterial ooze that may be visible if the stem is cut (Singh and Siddiqui, 2012). *Ralstonia solanacearum* and *R. pseudosolanacearum* cause bacterial wilt and are major destructive pathogens in tomato production (Prior et al., 2016). Bacterial spots of tomatoes are caused by different *Xanthomonas* spp., such as *X. vesicatoria* and *X. perforans*, and these species have been reported to reduce the yield of tomatoes. The weight of harvested fruit in one study was reduced by 52 % due to the defoliation of tomato leaves by *X. vesicatoria* (Reddy et al., 2012). Also, pathogenic fungi are the main infectious agents in crops, causing alterations during developmental stages including post-harvest (Dellavalle et al., 2011). These phytopathogens reduce both the quality and quantity of tomatoes, thus hindering agricultural production and the delivery of sustainable food.

Numerous strategies are employed globally for controlling plant diseases, including sanitation, crop rotation with non-host cover crops, use of chemicals, pathogen-free transplants and the use of resistant cultivars (Pradhanang et al., 2005). These methods are highly effective, however, some are prone to difficulties like the use of resistant cultivars which are limited in terms of location, climate and resistance to strains of the pathogen (Saddler, 2004). Pesticides have been universally considered the most efficient solution to control crop diseases. Streptomycin and copper-based compounds are effective antibiotics that have been used for controlling bacterial leaf spots and blights of tomatoes (Obradovic et al., 2008). Fungicides are efficient, pose less of a risk to crops, animals and the environment, and have been used successfully to protect plants against fungal attacks (Mancini and Romanazzi, 2014). However, excessive and unsystematic application of these synthetic chemicals is a threat to the environment and human health, and some phytopathogens have developed resistance to antibiotics, rendering them ineffective (da Silva et al., 2016). Moreover, the costs and contamination due to chemical residues in crops have necessitated the search for alternative methods for controlling plant diseases.

Plant-derived chemicals offer a feasible alternative to such problems, since most are safe for humans, animals and the environment. According to Chitwood (2002), the US Environmental Protection Agency requires fewer data to register phytochemicals than conventional pesticides, and registration costs are lower. Plants are rich in a variety of secondary metabolites that have several pharmacological effects in humans and animals. As reported by Pretorius et al. (2003), citing several researchers including Hutchings et al. (1996), many studies have been conducted on antimicrobial activity of South African plants against human pathogenic bacteria and fungi; however, few reports exist on activity against plant pathogenic microbes. Research on crop protection using plants in South Africa has gained momentum since then. Pretorius et al. (2003) reported antibacterial activity of 26 plants from South Africa which inhibited the growth of one or more of *Agrobacterium tumefaciens, Clavibacter michiganense* pv. *michiganense, Erwinia carotovora* pv. *carotovora, Pseudomonas solanacearum* and *Xanthomonas campestris* pv. *phaseoli*. Mdee et al. (2009) investigated the antifungal activity of extracts of invasive plants in South Africa, which exhibited some activity against *Penicillium janthinellum, P. expansum, Aspergillus niger, A. parasiticus, Colletotrichum gloeosporioides, Fusarium oxysporum, Trichoderma harzianum, Phytophthora nicotiana, Pythium ultimum* and *Rhizoctonia solani.* The methanolic extracts of *Monsonia burkeana* and *Moringa oleifera* reduced the growth of *Fusarium oxysporum* f.sp. *lycopersici in vitro* and wilt severity on tomato seedlings under greenhouse conditions (Hlokwe et al., 2018). Though studies on phytopathogenic microbes are increasing, exploration of South African flora is still lacking, hence the purpose of the present study was to investigate the potential of selected plants against bacterial and fungal disease of tomatoes. Plants were selected based on their activity against plant-parasitic nematodes (*Meloidogyne* spp.) (Makhubu, 2021) to determine if those plants also have antimicrobial activity against pathogens infecting tomato.

2. Materials and methods

2.1. Plant collection and extraction

2.1.1. Collection and identification

The fresh leaves of ten plants were collected at the University of Pretoria (Manie van der Schijff Botanical Garden, the Experimental Farm and the grounds of the Faculty of Veterinary Science) in spring months (August-September 2018). Voucher specimens were deposited in the H.G.W.J. Schweickerdt Herbarium, University of Pretoria (PRU). The species collected and their voucher specimen numbers are as follows: *Searsia lancea* (L.f.) F.A. Barkley (PRU 124364), *Acokanthera oppositifolia* (Lam.) Codd. (PRU 124363), *Cotyledon orbiculata* L. (PRU 0125286), *Curtisia dentata* (Burm.f.) C.A.Sm. (PRU 124361), *Leonotis leonurus* (L.) R. BR. (PRU 124359), *Clausena anisata* (Willd.) Hook.f. ex Benth. (PRU 124385), *Leucosidea sericea* Eckl. & Zeyh (PRU 124358), *Hippobromus pauciflorus* (L.f.) Radlk (PRU 124360), *Clerodendrum glabrum* E. Mey (PRU 124362) and *Lantana rugosa* Thunb. (PRU 124386).

2.1.2. Plant extraction

The collected leaf material was dried at room temperature in a well-ventilated room and ground to a fine powder in a Macsalab Mill (Model 2000 LAB Eriez). Succulent leaves of *C. orbiculata* were oven-dried at 45–50 °C until constant weight was reached. Twenty-five g of each leaf sample were separately extracted in 250 mL of distilled water, acetone and DCM/MeOH (1:1 v/v) (Minema Chemicals). The extraction was left at room temperature for 30 min and filtered through Whatman No. 1 filter paper. The procedure was repeated twice on the same plant material to exhaustively extract plant material and left overnight (24 h) during the final extraction. All the organic filtrates were concentrated in a rotary evaporator and water extracts were dried in front of a stream of cold air. Extracts were stored at 8 °C until use.

2.2. Antimicrobial experiments

2.2.1. Test microorganisms and growth conditions

Five pathogenic bacterial species: *Clavibacter michiganensis* subsp. *michiganensis* BD 1377, *Xanthomonas vesicatoria* BD 1349, *Xanthomonas perforans* BD 1346, *Ralstonia solanacearum* CBD 261, *Ralstonia pseudosolanacearum* BD 1443 and one fungal strain of *Fusarium oxysporum* f.sp. *lycopersici* PPRI 5457 were used in the study. These strains had previously been isolated from diseased tomato plants and were stored in the Plant Pathogenic and Plant Protecting Bacteria Culture Collection (verified by T. Goszczynska) and the National Collection of Fungi (verified by J.A. Venter) of the ARC-Plant Health and Protection (ARC-PHP, Roodeplaat), South Africa.

Purified colonies of *Ralstonia, Xanthomonas* and *Cmm* were inoculated into Mueller Hinton broth (beef infusions solids 2 g/l; starch 1.5 g/l: casein hydrolysate 17.5 g/l at pH 7.4 ± 0.2) and incubated at 28±2 °C for 24 h in an orbital shaker incubator with the latter incubated for seven days. *Xanthomonas perforans* was incubated at 28±2 °C for 10 days. *Fusarium* was inoculated into Potato Dextrose broth and incubated at 28 ± 2 °C for 48 h in an orbital shaker incubator.

2.2.2. Minimal inhibitory concentration (MIC) evaluation against bacterial and fungal strains

A serial microplate dilution method described by Eloff (1998a) was used to determine the minimal inhibitory concentrations of the crude extracts on the selected bacterial and fungal pathogens. The acetone and aqueous extracts were re-dissolved in their respective solvents while DCM/MeOH extracts were re-dissolved in 10 %

dimethyl sulfoxide (DMSO) to give a concentration of 10 mg/mL. For the antibacterial assay, inoculum suspension from overnight cultures was prepared by diluting the bacterial colonies with fresh Mueller Hinton broth to give a final concentration standardized to a McFarland standard No 1 (equivalent to 3×10^8 cfu/mL). In the antifungal assay inoculum, suspensions from overnight cultures was prepared similarly by diluting the fungal colonies with fresh Potato Dextrose broth to a final concentration of McFarland standard No 1 (equivalent to 3×10^8 cfu/mL). Sterile water (100 μL) was dispensed in each well of a 96-well microtitre plate and plant extracts (100 μL) were serially diluted two-fold down the columns. A 100 μL aliquot of bacterial or fungal culture was added to each well resulting in concentrations ranging from 2 500 μg/mL to 19.5 μg/mL. A similar two-fold serial dilution of streptomycin (Sigma) (starting concentration 2 000 μg/mL) was used as a positive control for the bacterial assay and amphotericin B (Sigma, 10 000 μg/mL starting concentration) was the positive control for the fungal assay. The solvent dilutions were used as negative controls. Plates were incubated at 28 °C in a humidified atmosphere for 24 h for the antibacterial assay, and 48 h for the antifungal assay. To measure growth, *p*-iodonitrotetrazolium violet (INT) (40 μL of a 0.2 mg/mL solution in sterile water) was added to each well and incubated further for 30 min (against bacterial strains), or 24 and 48 h (against fungal strains). The minimum inhibitory concentration was recorded as the lowest concentration where clear wells indicated inhibition of microbial growth, as reduction of INT to a red formazan product indicates growth. The experiment was done in triplicate and repeated three times. MIC values are presented as mean±standard deviation.

3. GC–MS chemical profiling

GC–MS analysis was carried out using a LECO Pegasus 4D GC-TOFMS (LECO Africa (Pty) Ltd., Kempton Park, South Africa) with a Rxi-5SilMS GC column (30 m x 0.25 mm ID x 0.2 μm film thickness) (Restek, Bellefonte, PA, USA). The following parameters were used: Injection volume of 1 μl, used splitless injection with splitless time set at 30 s; GC inlet was 250 °C; GC oven temperature programme was 40 °C (hold for 3 min) at 10 °C/min to 300 °C (hold for 5 min). Carrier gas used was UHP Helium (Afrox, South Africa) at 1 ml/min set at constant flow mode. Mass acquisition range was 40–550 Da MS transfer line at temperature 280 °C; Ion source temperature was 230 °C; MS solvent was delayed at 5 min. The ionisation energy was 70 eV in the electron ionisation mode (EI+); the voltage of the detector was 1750 V and the GC was operated at an acquisition rate of 10 spectra/ s. Tentative identification of the compounds was achieved by comparison of experimental mass spectra with reference spectra of the National Institute of Standards and Technology (NIST) library (version 2.2) and PubChem with the minimum similarity threshold for a match set at 85 % (Jenke et al., 2020; Wooding et al., 2020).

4. Results

4.1. Antimicrobial activity

The MIC values of plant extracts against bacterial phytopathogens are presented in Table 1. In this study, the activity of the plant extracts was categorised based on the recommendations of Eloff (2021), where MIC values were classified as: outstanding activity ≤20 μg/ml, excellent activity 21–40 μg/ml, very good activity 41–80 μg/ml, good activity 81–160 μg/ml, average activity 161–320 μg/ml, and weak activity > 320 μg/ml. Significant inhibition and a broader spectrum of antibacterial activity was observed with the acetone extracts, followed by DCM/MeOH extracts, while those prepared using water showed weak activity. Acetone and DCM/MeOH extracts of *L. sericea* and *S. lancea* demonstrated outstanding to good antibacterial activity inhibiting the growth of bacteria with MIC values ranging between 19.5 and 97.5 μg/mL. An outstanding MIC value of 19.5 μg/mL was attained by the acetone extract of *S. lancea* against *R. solanacearum* and *X. vesicatoria,* and the

Table 1
Minimum inhibitory concentration (MIC) values (μg/mL) of extracts from different plant species against test bacterial strains (mean ± SD).

Plant species	Extracts	*R. pseudosolanacearum*	*R. solanacearum*	*C.m.*subsp. *michiganensis*	*X. perforans*	*X. vesicatoria*
A. oppositifolia	H_2O	2 500 ± 0.00	2 500 ± 0.00	>2 500	2 500 ± 0.00	938 ± 0.44
	Ace	>2 500	2 500 ± 0.00	1 562 ± 1.33	2 500 ± 0.00	2 500 ± 0.00
	DCM/MeOH	1 250 ± 0.00	1 562 ± 1.33	2 500 ± 0.00	2 500 ± 0.00	938 ± 0.44
C. anisata	H_2O	2 500 ± 0.00	2 500 ± 0.00	2 500 ± 0.00	2 500 ± 0.00	2 500 ± 0.00
	Ace	1 563 ± 1.33	938 ± 0.44	1 563 ± 1.33	625 ± 0.00	625 ± 0.00
	DCM/MeOH	1 250 ± 0.00	2 500 ± 0.00	2 500 ± 00	2 500 ± 0.00	625 ± 0.00
C. glabrum	H_2O	1 563 ± 1.33	1 875 ± 0.88	938 ± 0.44	1 250 ± 0.00	2 500 ± 0.00
	Ace	2 500 ± 0.00	664 ± 0.83	176 ± 0.19	313 ± 0.00	2 500 ± 0.00
	DCM/MeOH	625 ± 0.00	391 ± 0.33	625 ± 0.00	938 ± 0.44	117 ± 0.06
C. orbiculata	H_2O	>2 500	>2 500	>2 500	>2 500	>2 500
	Ace	332 ± 0.41	313 ± 0.00	313 ± 0.00	391 ± 0.33	469 ± 0.22
	DCM/MeOH	625 ± 0.00	1 562 ± 1.33	2 500 ± 0.00	2 500 ± 0.00	781 ± 0.66
C. dentata	H_2O	2 500 ± 0.00	>2 500	>2 500	>2 500	2 500 ± 0.00
	Ace	1 250 ± 0.00	782 ± 0.66	469 ± 0.22	938 ± 0.44	1 875 ± 0.88
	DCM/MeOH	625 ± 0.00	625 ± 0.00	625 ± 0.00	1 250 ± 0.00	703 ± 0.77
H. pauciflorus	H_2O	2 500 ± 0.00	1 875 ± 0.88	2 500 ± 0.00	1 875 ± 0.88	>2 500
	Ace	1 093 ± 0.00	1 405 ± 0.22	235 ± 0.11	313 ± 0.00	391 ± 0.33
	DCM/MeOH	1 250 ± 0.00	2 500 ± 0.00	2 500 ± 0.00	2 500 ± 0.00	1 875 ± 0.88
L. *rugosa*	H_2O	1 875 ± 0.88	2 500 ± 0.00	2 500 ± 0.00	1 875 ± 0.00	1 875 ± 0.88
	Ace	937 ± 0.88	819 ± 1.05	469 ± 0.22	313 ± 0.00	156 ± 0.00
	DCM/MeOH	625 ± 0.00	176 ± 0.19	469 ± 0.22	938 ± 0.44	156 ± 0.00
L. *leonurus*	H_2O	469 ± 0.22	625 ± 0.00	938 ± 0.44	469 ± 0.22	469 ± 0.22
	Ace	469 ± 0.22	469 ± 0.22	938 ± 0.44	469 ± 0.22	235 ± 0.11
	DCM/MeOH	469 ± 0.22	469 ± 0.22	938 ± 0.44	1 875 ± 0.88	156 ± 0.00
L. *sericea*	H_2O	2 500 ± 0.00	1 875 ± 0.88	625 ± 0.00	625 ± 0.00	1 562 ± 1.33
	Ace	**19.5 ± 0.00**	332 ± 0.41	117 ± 0.06	235 ± 0.11	**19.5 ± 0.00**
	DCM/MeOH	**39 ± 0.00**	**29.3 ± 0.01**	469 ± 0.22	782 ± 0.66	**19.5 ± 0.00**
S. lancea	H_2O	>2 500	>2 500	>2 500	2 500 ± 0.00	>2 500
	Ace	97.5 ± 0.00	**19.5 ± 0.00**	58.5 ± 0.03	156 ± 0.00	**19.5 ± 0.00**
	DCM/MeOH	156 ± 0.00	48.8 ± 0.04	938 ± 0.44	782 ± 0.66	78 ± 0.00
Positive control	Streptomycin	12 ± 0.00	19.5 ± 0.00	10 ± 0.00	10 ± 0.00	10 ± 0.00

N.B: Ace: Acetone; H_2O: Water; DCM/MeOH: DCM/MeOH (1:1 v/v). Bold values refer to outstanding (≤20 μg/ml) and excellent (21–40 μg/ml) MICs.

Table 2
Minimum inhibitory concentration (MIC) values (μg/mL) of extracts from different plant species against *Fusarium oxysporum* f.sp. *lycopersici* (mean±SD) incubated for 24 and 48 h.

Plant species	Extracts	24 h	48 h
A. oppositifolia	H_2O	1 563 ± 1.33	1 875 ± 0.88
	Ace	>2 500	>2 500
	DCM/MeOH	1 563 ± 1.33	>2 500
C. anisata	H_2O	1 875 ± 0.88	2 500 ± 0.00
	Ace	1 563 ± 1.33	625 ± 0.00
	DCM/MeOH	2 500 ± 0.00	>2 500
C. glabrum	H_2O	2 500 ± 0.00	>2 500
	Ace	645 ± 0.86	782 ± 0.66
	DCM/MeOH	938 ± 0.44	1 250 ± 0.00
C. orbiculata	H_2O	>2 500	>2 500
	Ace	**39 ± 0.00**	156 ± 0.00
	DCM/MeOH	1 250 ± 0.00	2 500 ± 0.00
C. dentata	H_2O	>2 500	>2 500
	Ace	1 563 ± 1.33	1 875 ± 0.88
	DCM/MeOH	1 250 ± 0.00	>2 500
H. pauciflorus	H_2O	1 875 ± 0.88	2 500 ± 0.00
	Ace	1 250 ± 0.00	1 250 ± 0.00
	DCM/MeOH	1 250 ± 0.00	1 250 ± 0.00
L. *rugosa*	H_2O	938 ± 0.44	1 875 ± 0.88
	Ace	235 ± 0.11	469 ± 0.22
	DCM/MeOH	1 563 ± 1.32	>2 500
L. *leonurus*	H_2O	97.5 ± 0.08	469 ± 0.22
	Ace	2 500 ± 0.00	>2 500
	DCM/MeOH	782 ± 0.66	1 250 ± 0.00
L. *sericea*	H_2O	>2 500	>2 500
	Ace	782 ± 0.66	1 563 ± 1.33
	DCM/MeOH	>2 500	>2 500
S. lancea	H_2O	2 500 ± 0.00	>2 500
	Ace	156 ± 0.00	156 ± 0.00
	DCM/MeOH	938 ± 0.44	1 563 ± 1.33
Positive control	Amphotericin B	78 ± 0.00	313 ± 0.00

H_2O: Water; Ace: Acetone; DCM/MeOH: dichloromethane:methanol. Values in bold represent excellent activity (MIC = 21–40 μg/ml).

L. sericea DCM/MeOH extract on *X. vesicatoria,* as well as its acetone extract on *R. pseudosolanacearum* and *X. vesicatoria.* The acetone extract of *S. lancea* produced a similar MIC of 19.5 μg/mL to that of streptomycin against *R. solanacearum.* Excellent MIC values of 29.3 μg/mL and 39 μg/mL were attained with the DCM/MeOH extract of *L. sericea* against *R. solanacearum* and *R. pseudosolanacearum* respectively. Very good activity was observed with the DCM/MeOH extract of *L. sericea* against *R. solanacearum* and *X. vesicatoria* with MIC values of 49 and 78 μg/mL respectively. *Clavibacter michiganensis* subsp. *michiganensis* was poorly inhibited by all the plant extracts, with the exception of acetone extracts of *S. lancea* and *L. sericea* which had good MIC values of 59 and 117 μg/mL respectively.

The MIC results of the extracts against *Fusarium oxysporum* f.sp. *lycopersici* are presented in Table 2. Most extracts had weak activity, and further incubation resulted in the growth of fungi in most extracts. *Searsia lancea* acetone extract showed good activity with a MIC value of 156 μg/mL at all incubation times while that of *L. sericea* showed weak activity with MIC value of 782 μg/mL after 24 h. Further incubation resulted in MIC = 1 563 μg/mL, thus implying low levels of inhibition of *F. oxysporum* f.sp. *lycopersici* growth. The excellent activity was observed with the acetone extracts of *C. orbiculata* with MIC value of 39 μg/mL against *F. oxysporum,* lower than the MIC of amphotericin B, followed by the water extract of *L. leonurus* which showed good activity with MIC = 97.5 μg/mL. However, further incubation resulted in the growth of fungi with MIC of 156 and 469 μg/mL respectively.

4.2. Chemical profiling

The chromatogram analysis of the acetone and DCM/MeOH extracts indicated major peaks which were similar in most plants and were identified as 9-octadecenamide, (Z)-, octadecanoic acid, dodecanamide, hexanamide, hexadecanoic acid and hexanoic acid. Only constituents with a high percentage in the extract were reported with similarity above 85 % and unidentified components with low similarity percentage were not reported (Table 3). The 9-octadecenamide was found to be the most highly concentrated constituent in *L. sericea* with 45 % and 41 % in DCM/MeOH and acetone extracts, respectively. The high quantity of 9-octadecenamide was also detected in the DCM/MeOH extracts of *S. lancea* (37 %), *C. dentata* (33 %), *A. oppositifolia* (28 %), *S. lancea* acetone extract (26 %), and the acetone extracts of *H. pauciflorus* (26 %) and *C. orbiculata* with 26 %, and lastly the DCM/MeOH extract of *H. pauciflorus* with 25 %. The second highest constituent with a percentage area of 18 % was thunbergol and hexanamide detected in the DCM/MeOH extracts of *H. pauciflorus* and *L. sericea*, respectively. It was observed that the extracts contained similar compounds, though the quantity of such compounds was not the same. *Hippobromus pauciflorus* DCM/MeOH extract indicated a high percentage of hexanamide (11 %) visible at a retention time of 1078.2 s while the acetone extract had 9 % of the same compound, showing at 1183.5 s. Dodecanamide in the same plant was high in the acetone extract with 6 % detectable at 1202.4 s while in the DCM/MeOH extract it was 4 % at 1204.1 s. The constituents lower than 5 % in the plants were n-hexadecanoic acid, 9,12,15-octadecatrienal, dotriacontane, eicosane, benzothiazol, octadecanoic acid, neophytadiene, 2,5-dimethyl-2-(2-tetrahydrofuryl) tetrahydrofuran, α-amyrin, 1-iodo-2-methylundecane and dotriacontane.

5. Discussion

Soilborne pathogens have a great impact on quality and quantity of crops, and antimicrobial resistance has heightened the economic consequences of various diseases. Tomatoes are affected by a large number of pathogenic organisms, and efforts are being made to reduce the impact of disease on this important crop. In developing a disease reduction strategy, plant-based preparations may potentially be used to assist in controlling microbial pathogens. Plant extracts with MIC values less than 100 μg/mL are considered to be pharmacologically significant (Eloff, 2004).

The good antibacterial activity (lower MIC values) shown by *S. lancea* in the present study against tested bacterial strains is supported by other researchers working on both human and plant pathogenic microbes. Pretorius et al. (2003) reported the antibacterial activity of *S. lancea* using the agar diffusion method. In their study, a mean zone inhibition of 7.0 mm against *Clavibacter michiganensis* subsp. *michiganensis* and 8.0 mm against *Pseudomonas solanacearum* (currently known as *Ralstonia solanacearum*) was demonstrated by the *S. lancea* methanolic extract. In the current study with the MIC method and different solvents used, the acetone extract inhibited *R. solanacearum* and *C. michiganensis* subsp. *michiganensis* with MIC values of 19.5 μg/mL and 58.5 μg/mL respectively. In support of its broad antimicrobial activity, Mayekiso et al. (2009) reported the antibacterial activity of *S. lancea* on *Staphylococcus aureus, Enterococcus coli, E. faecalis, Pseudomonas aeruginosa, Mycobacterium smegmatis* and *M. fortuitum*. Aqueous and ethanolic stem bark extracts of *S. lancea* also had good activity against *S. aureus, Streptococcus pyogenes, Bacillus cereus, Salmonella typhi, Shigella sonnei* and *Escherichia coli;* however, there was no activity in the leaf extracts (Obi et al., 2003). According to Gundidza et al. (2008), compounds such as α-pinene, benzene, δ−3-carene, isopropyl toluene, and trans-caryophyllene in *S. lancea* were found to have medicinal properties, such as antibacterial, antifungal and antioxidant activities.

Leucosidea sericea had good activity against four phytopathogens with strong MIC values in the current study and has been previously reported to have antimicrobial activity against human pathogenic bacteria and fungi such as *S. aureus, E. coli, B. subtilis, S. sonnei, S. typhimurium, Klebsiella pneumoniae, P. aeruginosa, S. typhi* and *Candida albicans* (Bosman et al., 2004; Aremu et al., 2010; Pitso and

Table 3
Chemical profiling of acetone and DCM/MeOH extracts from ten South African plants.

Plant name	Extract	Chemical name	MW	MF	CAS	Similarity%	Retention time (s)	Area%
A. oppositifolia	Acetone	Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	88	1075.6	9
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1184.2	16
	DCM/MeOH	Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	91	1075.5	9
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1183.4	28
C. anisata	Acetone	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	57–10–3	92	961.3	5
		Octadecanoic acid (stearic acid)	284	$C_{18}H_{36}O_2$	57–11–4	93	1095.1	7
		9,12,15-Octadecatrienal	262	$C_{18}H_{30}O$	26,537–71–3	86	1346	3
		Dotriacontane	450	$C_{32}H_{66}$	544–85–4	94	1598.1	3
		Eicosane	282	$C_{20}H_{42}$	112–95–8	90	1346.8	3
	DCM/MeOH	Benzothiazole	135	C_7H_5NS	95–16–9	95	328.3	5
		Octadecanoic acid	284	$C_{18}H_{36}O_2$	123–94–4	91	1093.6	5
C. glabrum	DCM/MeOH	Hexanamide	115	$C_6H_{13}NO$	628–02–4	87	1076.7	11
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1183.7	22
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	92	1202.9	7
C. orbiculata	Acetone	Neophytadiene	278	$C_{20}H_{38}$	504–96–1	88	846.2	3
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	93	1186.4	25
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	93	1204.5	8
	DCM/MeOH	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1183.3	22
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	89	1202.2	7
		Dotriacontane	450	$C_{32}H_{66}$	544–85–4	90	1667.9	9
C. dentata	Acetone	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1183.5	14
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	94	1202.5	4
	DCM/MeOH	Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	92	1076.3	13
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1184.2	33
H. pauciflorus	Acetone	Hexanamide	115	$C_6H_{13}NO$	628–02–4	86	1183.5	9
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	92	1202.4	6
		Thunbergol	290	$C_{20}H_{34}O$	25,269–17–4	85	1661.5	18
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1183.5	26
	DCM/MeOH	Hexanamide	115	$C_6H_{13}NO$	628–02–4	88	1078.2	11
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	93	1185.8	25
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	95	1204.1	4
L. rugosa	Acetone	Octadecanoic acid	284	$C_{18}H_{36}O_2$	64,165–21–5	93	1093.6	5
		Benzothiazole	135	C_7H_5NS	95–16–9	96	327.1	3
		2,5-Dimethyl-2-(2-tetrahydrofuryl) tetrahydrofuran	170	$C_{10}H_{18}O_2$	0–00–0	85	615.2	4
		α-Amyrin	426	$C_{30}H_{50}O$	638–95–9	86	1680.3	3
L. leonurus	DCM/MeOH	Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	90	1080.5	6
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1188.1	20
L. sericea	Acetone	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1196.8	41
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	90	1215.3	14
	DCM/MeOH	Hexanamide	115	$C_6H_{13}NO$	628–02–4	88	1076.1	18
		9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1184	45
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	90	1202.7	13
S. lancea	Acetone	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	92	1184.7	26
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	95	1202.4	7
		1-Iodo-2-methylundecane	296	$C_{12}H_{25}I$	73,105–67–6	92	1482.9	4
		Dotriacontane	450	$C_{32}H_{66}$	544–85–4	90	1578.5	4
	DCM/MeOH	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	301–02–0	91	1183.3	37
		Dodecanamide	199	$C_{12}H_{25}NO$	1120–16–7	91	1202.2	10
		Dotriacontane	450	$C_{32}H_{66}$	544–85–4	91	1482.7	5

MW- Molecular weight; MF- Molecular formula.

Ashafa, 2015). *Cotyledon orbiculata* leaf extract was found to have antimicrobial activity against GMRSA, *Staphylococcus epidermidis, P. aeruginosa, C. albicans, Propionibacterium acnes* and *Microsporum canis* at varying MIC values of 0.25–1 mg/mL (Mabona et al., 2013). The good antimicrobial activity shown by acetone extracts in the present study could be because acetone can extract compounds with a wide range of polarities, is volatile, miscible with water, and has low toxicity to bacteria and fungi (Eloff, 1998b). Acetone is usually preferred as an extractant for plant material because it extracts both polar and non-polar components (Masoko and Eloff, 2006) and it has low toxicity (Eloff et al., 2007).

The most susceptible organisms in the present study were *R. pseudosolanacearum, R. solanacearum* and *X. vesicatoria. Ralstonia* and *Xanthomonas* species are associated with roots of tomatoes, and since nematodes cause injury to the roots of host plants, the resultant damage with signs of gall formation, wilting symptoms as well as spots on leaves reduce the quality and quantity of the tomato yield (Singh and Siddiqui, 2012). The weak activity shown by *S. lancea* and L. *sericea* extracts against *F. oxysporum* f.sp. *lycopersici* is supported by the report of Heisey and Gorham (1992) cited in Pretorius et al. (2003), stipulating that plant pathogenic fungi are more resistant to natural extracts from plants than plant pathogenic bacteria. The good activity shown by plants in the present study will assist in mitigating the effects of bacterial wilt, spot, and canker as well as wilting caused by *Fusarium* species, which are caused by the pathogens included in this investigation. Besides the *in vitro* experiments perfomed in the present study, pathogen assays in plants (*in vivo* studies) should be performed to study the viability of these extracts against plant pathogens. A wider range of fungi affecting tomatoes will probably have to be tested to determine if *H. pauciflorus, L. sericea, A. oppositifolia, S. lancea, C. dentata* and *C. anisata* might have activity and to come to a definite conclusion regarding the antifungal status of *C. orbiculata* and *L. leonurus.*

Gas chromatography-mass spectrometry (GC–MS) represents one of the oldest and most successful hyphenations of separation techniques to a mass spectrometer (Wiles, 2011). It is the most common application for characterization of natural products for their chemical composition to aid antimicrobial activity studies, toxicological analyses, chemical ecological studies, the characterization of materials, and pharmaceutical/drug monitoring and clinical analyses to mention some (Sichilongo et al., 2012). The profiled chemicals based on GC–MS analysis indicated the presence of various chemicals, including fatty acids, amides and sterols. According to Seow et al. (2012), the presence of a hydroxyl (OH) group of aromatic compounds and fatty acids in a plant allows such chemicals to enter the membrane of the bacteria by use of dispersion forces, thereby causing disturbances of the fluidity of the cell membranes and affecting the growth of the bacteria. The presence of various fatty acids in the acetone and DCM/MeOH extracts such as n-hexadecanoic acid (palmitic acid), dodecanamide (fatty amide of lauric acid), 9-octadecenamide, (Z)-(amide derived from the fatty acid oleic acid), and octadecanoic acid (stearic acid) in the investigated plants most likely contributed to the antimicrobial activity reported in this study. Octadecanoic acid has been reported to have antibacterial and antifungal potential (Gopalakrishnan and Udayakumar, 2014). Dos Reis et al. (2019) reported 75.83 % of 9-octadecenamide in the ethyl acetate extract of *Diaporthe schini*, and the extract was active against *Staphylococcus epidermidis, Enterobacter derogenes, Klebsiella pneumoniae* and *Candida krusei* (Gopalakrishnan and Udayakumar, 2014). Other than fatty acids and amides, the majority of the isolated antimicrobials belonging to the phenolic, terpene, alkaloid and polypeptide chemical groups have been reported to exhibit multiple mechanisms of action against disease causing microorganisms (Singh, 2017). The presence of these compounds detected in the present study, and also those that were undetected or occur in lesser amounts, may contribute to the antimicrobial activity of the plants in synergism with other major compounds detected, and those with aromatic regions and fatty acids could assist in inhibiting the growth of the tested phytopathogens.

6. Conclusion

The findings of this study demonstrated that *L. sericea* and *S. lancea* extracts are potential alternatives that can be further investigated as broad-spectrum bactericidal agents with their promising activity against most tested bacteria in the present study. The profiled chemicals in the plants, even those occurring in low amounts, are likely to contribute synergistically to the activity shown against the plant pathogens tested. Though many studies are focussed on investigating the activity of plant extracts and plant-derived compounds on human and animal pathogens, such plants can also be tested against plant pathogens with the aim of developing alternatives to conventional chemicals. Therefore, there is a need to further investigate the active plants identified in this study for efficacy against other microbes and their mechanisms of action. Additionally, it is recommended to test the efficacy of the plant extracts under field conditions, and to identify the phytochemicals responsible for the plant antimicrobial activity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

F.N. Makhubu: Conceptualization, Data curation, Investigation, Formal analysis, Writing – original draft. **M.C. Khosa:** Conceptualization, Resources, Supervision, Writing – review & editing. **L.J. McGaw:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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