applied sciences

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In Vitro Antibacterial Activity of Selected South African Plants against Drug-Resistant Staphylococci Isolated from Clinical Cases of Bovine Mastitis

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Featured Application: Natural products from plant extracts have demonstrated interesting biological activities. With the rise in antimicrobial resistance, plants may provide complementary or alternative approaches to combating diseases, such as mastitis, assisting in sustainably reducing the burden of microbial infections.

Abstract: Bovine mastitis in dairy livestock production is a serious economic problem causing milk production losses and increased management costs. *Staphylococcus aureus* and non-aureus staphylococcus (NAS) are important causes of bovine mastitis. Antimicrobial resistance can limit the control of mastitis pathogens. Antibacterial and cytotoxic activities of acetone and ethanol extracts of nine South African plants were determined against reference and drug-resistant staphylococci isolated from clinical cases of bovine mastitis. The nine plants, namely, *Antidesma venosum*, *Elaeodendron croceum*, *Erythrina caffra*, *Indigofera frutescens*, *Pleurostylia capensis*, *Searsia lancea*, *Searsia leptodictya*, *Trichilia emetica*, and *Ziziphus mucronata*, were chosen for research material. The antibacterial activity of extracts was determined using a serial microdilution method, while a tetrazolium-based assay was used to determine their cytotoxicity against Vero cells. The values of antibacterial minimum inhibitory concentration (MIC) obtained were promising, with MIC ranging between 0.01 and 1.41 mg/mL. Generally, acetone extracts of most plants had better activity than their ethanol counterparts. The *S. aureus* strains were more susceptible to all the extracts than the NAS strains. The plant extracts' half-maximal toxicity (LC_{50}) was higher than 0.02 mg/mL, which is the recommended cytotoxic cut-off concentration. The ethanol extract of *E. caffra*, which showed better MIC values than its acetone extract, had the highest mean selectivity index (SI) of 8.30. Among the plants tested, *S. lancea* exhibited the most potent antibacterial activity, suggesting its potential usefulness as a broad-spectrum antibacterial agent against staphylococci associated with bovine mastitis, at relatively non-cytotoxic concentrations. Therefore, it is recommended for further investigation.

Keywords: mastitis; antibacterial; cytotoxicity; staphylococci; drug-resistance

Citation: Akinboye, A.O.; Famuyide, I.M.; Petzer, I.-M.; McGaw, L.J. In Vitro Antibacterial Activity of Selected South African Plants against Drug-Resistant Staphylococci Isolated from Clinical Cases of Bovine Mastitis. *Appl. Sci.* **2023**, *13*, 5560. https://doi.org/10.3390/app13095560

Academic Editor: Antonio Valero

Received: 12 April 2023
Revised: 24 April 2023
Accepted: 26 April 2023
Published: 29 April 2023

1. Introduction

Dairy production systems provide milk with high content of carbohydrates, fats, vitamins, and minerals for the growing human population [1]. Several factors can cause milk production to fall short of the demand from the population, and one of the most common of these factors is mastitis [2]. The prevalence, as well as the economic impact of bovine mastitis, poses serious health problems and challenges of an economic nature in the bovine dairy industry worldwide [3]. Mastitis reduces productivity, and the economic effectiveness of dairy farms is adversely affected by decreased milk yield and returns,

as well as rising treatment costs [4]. The disease is responsible for economic losses of approximately USD 35 billion worldwide [5]. According to economic cost estimates, mastitis leads to a loss of approximately 70% of the total economic losses, mainly due to decreased milk production [6]. Other costs of mastitis are incurred from treatment, additional labor, decreased milk quality, and increased risk of culling with increased replacement cost [6].

Bovine mastitis is an inflammatory reaction of the mammary parenchyma that is usually caused by physical or microbial factors [7]. The severity of mastitis can be categorized into two types: clinical mastitis and subclinical mastitis, with clinical mastitis being divided into three levels of severity. Level 1 is identified by the secretion of abnormal milk, such as the presence of flakes or watery consistency. In contrast, Level 2 is characterized by changes in the milk and signs of inflammation in the udder, such as redness, swelling, hardness, and pain [8]. In Level 3, the two latter signs are present, as well as systemic signs in the animal that can, in some cases, be fatal. In contrast, subclinical mastitis is characterized by an absence of visible changes in the udder and milk, despite an increase in somatic cell count (SCC) [7]. It can be inferred that reduced milk production can be attributed to the decrease in the number and activity of alveoli, which is a consequence of the damage inflicted on mammary tissue.

Pathogens responsible for mastitis, including bacteria, fungi, and viruses [9], are broadly classified as host-adaptive or environmental in nature. Host-adaptive pathogens, which include *Staphylococcus aureus* [10] (*S. aureus*), certain non-aureus staphylococci (NAS), some *Streptococcus dysgalactiae* and *Streptococcus agalactiae*, are organisms that can adapt to surviving within the mammary parenchyma of the host, causing subclinical infections [11]. Environmental pathogens, such as members of the Enterobacteriaceae (particularly *E. coli*), *Streptococcus uberis*, and non-aureus staphylococci (NAS) [7], are opportunistic invaders of the mammary gland, and they are not adapted to survive within the host as is the case with adapted pathogens. However, they can invade and multiply within the mammary gland and stimulate the host-response system [8]. In South Africa, Petzer et al. in their 2009 review [7] suggested that *S. aureus* remains a major pathogen of bovine mastitis. Isolation of *Staphylococcus* spp. is a common occurrence in both clinical and subclinical mastitis cases, with NAS showing a rising trend, and antibacterial resistance reportedly common among these species. Certain isolates have been found to be resistant to as many as six commonly used antibiotics, highlighting the growing concern of treatment failure and the resulting increase in the cost of treating bovine mastitis.

The wide usage of antimicrobial agents for the treatment of pathogens found in mastitis in veterinary medicine is plagued with limited success due to the increasing prevalence of resistance to commonly used antimicrobial agents [12]. Most antibiotics (particularly β-lactams) used in mastitis treatment are reported to be ineffective against *Staphylococcus aureus*, which is capable of releasing toxins that compromise the natural defences of the udder [13]. In a recent report, 90% of NAS strains isolated from mastitis milk in South Africa exhibited resistance to at least one antibacterial agent, while 51% showed multidrug resistance [14]. Plant-derived natural products have been established as effective models for the development of new drugs since they have shown a range of useful biological activities. The use of natural plant products has increasingly become a recognized treatment for several ailments, and this is one of the rapidly growing sectors of the agribusiness industry [15]. Given the rising prevalence of *Staphylococcus* species and their growing resistance to commonly used antibiotics, there is a global effort to investigate natural products as potential sources of new and safer drugs [7,14].

South Africa is home to a diverse flora used for phytotherapeutic purposes, especially in traditional medicine, while some scientific rationale for their uses has been documented [16]. There are various local and global initiatives currently underway to investigate the botanical resources of southern Africa. The aim of these efforts is to analyze indigenous plants in order to identify potentially beneficial pharmacological compounds [17]. It has been cautioned that in cases where plants have been used therapeutically for many years,

it is likely that researchers assume that they are safe and exclude evaluating toxicological aspects of the traditional medicinal plant [17]. However, together with bioactivity screening for useful compounds, toxicity testing in vitro is a crucial aspect of preliminary safety evaluations of plant-derived extracts and compounds before recommending them for continued development and commercialization [18].

This study aimed to investigate the extracts of nine selected South African plants for their antibacterial activity against drug-resistant strains of *S. aureus* and NAS isolated from clinical cases of bovine mastitis. The cytotoxic effects of extracts of three plants selected based on their unique antibacterial activities were also determined to evaluate their antibacterial efficacy.

2. Materials and Methods

2.1. Plant Collection and Identification

The selection of plants for the current study was based on their known antimicrobial activity, chemotaxonomic relationships to plant species with antibacterial activity, availability, and/or the existence of traditional uses against infectious diseases. The leaves of the plants (Table 1) used in this study were harvested from the Manie van der Schijff Botanical Garden at the University of Pretoria, Onderstepoort campus of the University of Pretoria and the Lowveld National Botanical Garden, Nelspruit, Mpumalanga. Herbarium voucher specimens were prepared and deposited in the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria or in the National Herbarium (SANBI) Pretoria (PRE), South Africa.

Table 1. Selected plants and their herbarium accession numbers.

Family	Plant Species	Accession Numbers
Anacardiaceae	*Searsia leptodictya* (Diels) T.S.Yi, A.J.Mill. & J.Wen	PRU 128116
Anacardiaceae	*Searsia lancea* (L.f.) F.A.Barkley	PRU 128113
Celastraceae	*Elaeodendron croceum* (Thunb.) DC.	PRE 1004265
Celastraceae	*Pleurostylia capensis* (Turcz.) Loes.	PRE 1004260
Fabaceae	*Erythrina caffra* Thunb.	PRU 128360
Fabaceae	*Indigofera frutescens* L.f.	PRU 128111
Meliacae	*Trichilia emetica* Vahl	PRU 128115
Phyllanthaceae	*Antidesma venosum* E.Mey. ex Tul.	PRU 128361
Rhamnaceae	*Ziziphus mucronata* Willd. ssp. *mucronata*	PRU 128112

2.2. Storage and Grinding

Healthy leaves were harvested in open-mesh, loosely woven bags and dried indoors at room temperature under ventilated conditions. Dried leaves were ground to a fine powder using a Janke and Künkel Model A10 mill. The powders were stored in tightly closed glass containers in the dark at room temperature. The dried material was used since there are fewer problems associated with large-scale extraction of dried plant material compared to fresh plant material, and dried material may retain its biological activity for many decades [18].

2.3. Plant Extraction

The plant material was extracted using acetone and ethanol. Acetone is generally considered the most suitable solvent for plant extraction, as it can extract compounds with a broad range of polarities, is non-toxic to bioassay systems, and can be easily removed from extracts [19,20]. Ethanol was selected based on the preference for less-flammable solvents in commercial usage. Each sample of ground dry leaves (4 grams) was separately extracted with 40 mL of each solvent. The mixture was sonicated for 20 min, vigorously shaken, and then poured into a 50 mL polyester centrifuge tube. The tube was centrifuged at 4000× *g* for 10 min (using a Hettich Centrifuge, Roto-fix 32 A, Labotec, Johannesburg, South Africa). The supernatant was collected and filtered through Whatman No. 1 filter paper into pre-weighed glass vials. The extracts were concentrated by drying under a

stream of cold air. The dried extracts were weighed, and the yields were calculated by dividing the mass extracted by the initial mass.

2.4. Bacterial Isolates

For this study, a total of 12 strains of *Staphylococcus* were used: Eight strains of *S. aureus* and four strains of non-aureus staphylococcus (NAS), as shown in Table 2. These strains were isolated from composite milk samples of clinical mastitis cases that were submitted to the Onderstepoort Milk Laboratory at the Faculty of Veterinary Science, University of Pretoria, in 2019. To isolate the bacteria, suspected milk samples were streaked on bovine blood tryptose agar plates from Oxoid, Quantum Biotechnologies (Pty) Ltd., Krugersdorp, South Africa. The plates were then incubated aerobically at 37 °C (±1 °C) for 24–48 h. Presumptive *Staphylococcus* spp. colonies were initially identified based on phenotypic morphology and biochemical tests [21]. The *Staphylococcus* isolates were confirmed using Staph API (Biomerieux, South Africa (Pty) Ltd., Midrand, South Africa). All *Staphylococcus* isolates were also subjected to matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) identification as previously described [22]. Single pure colonies were transferred onto MALDI plates (Sigma Aldrich, St. Louis, MO, USA) in duplicate and covered with alpha-cyano-4-hydroxycinnamic acid in an organic solution (50% acetonitrile and 2.5% tri-fluoro-acetic acid). The preparation was crystallized by air drying at room temperature. Flex Control software (Bruker Daltonics, Billerica, MA, USA) recorded spectra sets for bacterial identification. MALDI Biolayer 3.0 software (Bruker Daltonics) with an integrated pattern-matching algorithm was used to compare generated peak lists against the reference library and a score was generated based on similarity. A commercial strain of *S. aureus* (ATCC 29213) was included as a reference strain for the bioassays.

Table 2. Crude extract yield of the selected plants.

Plants	% Acetone Extract Yield (g/g)	% Ethanol Extract Yield (g/g)
Antidesma venosum	1.92	3.28
Elaeodendron croceum	4.96	8.12
Erythrina caffra	5.35	2.68
Indigofera frutescens	1.68	6.87
Pleurostylia capensis	5.11	6.80
Searsia lancea	5.49	10.75
Searsia leptodictya	1.98	4.00
Trichilia emetica	2.79	6.99
Ziziphus mucronata	2.80	6.04

g/g = gram/gram.

2.5. Analysis of Extracts by Thin-Layer Chromatography (TLC)

To obtain thin-layer chromatography (TLC) fingerprints, crude acetone and ethanol extracts of *Antidesma venosum*, *Erythrina caffra*, and *Searsia lancea* were qualitatively screened using an established protocol [23]. The screening involved eluting 100 µg of each extract, which was loaded on a 1 cm wide band of aluminum-backed silica gel plates, with three different solvent systems of diverse polarities. The solvent systems used were benzene: ethanol: ammonium hydroxide (18:2:0.2, BEA, non-polar, basic), chloroform: ethyl acetate: formic acid (5:4:1, CEF, intermediate polarity, acidic), and ethyl acetate: methanol: water (40:5.4:5, EMW, polar, neutral). The TLC plates were marked with visible bands under white light and ultraviolet light (254 nm and 360 nm wavelengths, Camag universal UV light lamp TL-600). Freshly prepared vanillin (0.1 g vanillin, 28 mL methanol, 1 mL sulphuric acid) spray reagent was applied to the plates, which were then heated to 110 °C to achieve optimal color development.

2.6. Antibiotic Susceptibility Testing

The modified disc diffusion method (Kirby–Bauer method) was used to perform antimicrobial susceptibility testing of 12 isolates and *S. aureus* ATCC 29213 (control) against a panel of 10 drugs on Mueller–Hinton agar. This was carried out following the guidelines established by the Clinical and Laboratory Standards Institute [24]. The 10 antimicrobial drugs tested were ampicillin (AMP) (10 µg), chloramphenicol (C) (30 µg), tetracycline (TE) (30 µg), doxycycline (DO) (30 µg), amoxicillin + clavulanic acid (AMC) (30 µg), amikacin (AK) (30 µg), colistin sulphate (CT) (10 µg), trimethoprim + sulfamethoxazole (SXT) (25 µg), gentamicin (CN) (10 µg), and ceftiofur (EFT) (30 µg). The diameter of the zone of inhibition was used to classify the isolates as sensitive, intermediate, or resistant [24]. For analysis purposes, isolates that exhibited intermediate susceptibility were considered resistant. Isolates that were resistant to at least one antimicrobial drug were classified as "resistant", while those that were resistant to three or more antimicrobial groups were defined as "multidrug-resistant" [25].

2.7. Antibacterial Activity Assays

2.7.1. Qualitative Antibacterial Assay by TLC Bioautography

To prepare thin-layer chromatograms of acetone and ethanol extracts of *Antidesma venosum*, *Erythrina caffra*, and *Searsia lancea*, the same method as described in Section 2.5 above was used, with the exception that the plates were not sprayed with vanillin. After the eluents were removed, each plate was sprayed with an actively growing suspension of a *S. aureus* isolate (STA3), a NAS isolate (NAS D), or an ATCC (29213) strain of *S. aureus* that had been cultured for 18–24 h at 37 °C. The moist plates were allowed to dry slightly and then incubated at 37 °C in a closed plastic humidified sterile container for 24 h to promote bacterial growth on the plates. After incubation, the plates were sprayed with 2 mg/mL of freshly prepared p-iodonitrotetrazolium (INT) violet (Sigma) in sterile distilled water and incubated further for 1–2 h to enable the development of clear zones against a purple-red background. This indicated that the compounds separated on the chromatograms inhibited bacterial growth [26].

2.7.2. Quantitative Antibacterial Assay (Minimum Inhibitory Concentration Assay)

To determine the minimum inhibitory concentration (MIC) of crude plant extracts against eight isolates of S. aureus, four isolates of NAS, and one ATCC 29213 strain of *S. aureus*, a simple, reproducible, low-cost, and sensitive serial dilution microplate method [27] was utilized. The bacterial cultures were adjusted to a McFarland standard of 1 and grown overnight in MH broth. The dried extracts were dissolved in acetone to a concentration of 10 mg/mL, and 100 µL aliquots were added to the first well of a sterile 96-well microtiter plate containing 100 µL of water, which was serially diluted with sterile distilled water. A hundred microliters of appropriately adjusted bacterial cultures were then added to each well. The bacteria were exposed to final extract concentrations of 2.5, 1.25, 0.63, 0.32, 0.16, 0.08, 0.04, and 0.02 mg/mL. Gentamicin and acetone served as positive and negative controls, respectively. The final concentration of acetone used in the bioassays is not toxic to microorganisms [28]. The microplates were incubated at 37 °C for 18–24 h, and the MIC values were determined visually as the lowest concentration that inhibited growth.

The total activity (mL/g) of the extracts was calculated by dividing the mass in mg extracted from 1 g of plant material by the MIC in mg/mL, considering the maximum volume of solvent that can be used to dilute the mass extracted from 1 g of the plant with retention of antibacterial activity. This provides a measure of the efficacy of antibacterial compounds [29].

2.8. Cytotoxicity Assay

The 3-(4,5-dimethyl thiazolyl-2)-2.5-diphenyltetrazolium (MTT) assay was utilized to determine the cytotoxicity of specific plant extracts against Vero monkey kidney cells [30]. The plants selected for the study were *S. lancea*, *E. caffra*, and *A. venosum*, all of which

exhibited MIC values below 0.1 mg/mL against all the microorganisms tested. The Vero monkey kidney cells were cultivated in MEM supplemented with 0.1% gentamicin and 5% fetal calf serum, and a cell suspension of 5×10^4 cells/mL was added to the wells of columns 2–11 of a sterile 96-well microtitre plate. The plates were then incubated for 24 h at 37 °C in a 5% CO_2 incubator to allow the cells to adhere and reach exponential growth. The plant extracts at different concentrations in MEM were added to the plates in quadruplicate, and the microtitre plates were further incubated for 48 h at 37 °C in a 5% CO_2 incubator with the plant samples. Positive controls (doxorubicin chloride, Pfizer Laboratories) and untreated cells were also included in the assay. After incubation, the contents of each well were aspirated, and the cells were washed with PBS and replaced with fresh MEM. Then, 30 µL of MTT stock solution (5 mg/mL in PBS) was added to all the wells, and the plates were incubated for a further 4 h at 37 °C. The medium in each well was then carefully removed, and the cells were washed with PBS before adding 50 µL of DMSO to dissolve the MTT formazan crystals. The plates were gently shaken to dissolve the MTT solution, and the amount of MTT reduction was immediately measured by detecting absorbance in a microplate reader at a wavelength of 540 nm and a reference wavelength of 630 nm. The LC_{50} values were calculated as the concentration of plant samples resulting in a 50% reduction in absorbance compared to untreated cells, which correlated to killing 50% of the cells.

2.9. Statistical Analysis

Experimental results were expressed as mean ± standard error of the mean (SEM) of at least three replicates. Data were collated and analyzed using Microsoft Excel Version 2010.

3. Results

3.1. Plant Extract Yield

Varied yields of crude extracts were acquired from the nine plants through acetone and ethanol extraction (Table 2). Among the acetone-extracted plants, *Searsia lancea* had the highest yield (5.49%) followed by *Erythrina caffra* (5.35%), while *Indigofera frutescens* gave the lowest yield (1.68%). Among the ethanol extracts, *Searsia lancea* yielded the highest (10.75%) followed by *Elaeodendron croceum* (8.12%), and *Erythrina caffra* yielded the lowest (2.68%).

3.2. Bacterial Identification

Table 3 displays the phenotypic characteristics of the bacterial isolates. The NAS strains' species identities were confirmed, but more significantly, STA7 and STA8, which were initially believed to be NAS strains, were later identified as strains of *S. aureus* and labeled accordingly.

Table 3. Species names of the test bacteria.

Strain Codes	Species Names
ATCC 29213	*Staphylococcus aureus*
STA1	*Staphylococcus aureus*
STA2	*Staphylococcus aureus*
STA3	*Staphylococcus aureus*
STA4	*Staphylococcus aureus*
STA5	*Staphylococcus aureus*
STA6	*Staphylococcus aureus*
STA7	*Staphylococcus aureus*
STA8	*Staphylococcus aureus*
NAS A	*Staphylococcus chromogenes*
NAS B	*Staphylococcus haemolyticus*
NAS C	*Staphylococcus chromogenes*
NAS D	*Staphylococcus chromogenes*

ATCC = American type culture collections, STA = *Staphylococcus aureus*, NAS = Non-aureus staphylococcus.

3.3. Antibacterial Susceptibility

The antibacterial susceptibility of the bacterial isolates is shown in Table 4. All the organisms were resistant to at least one of the antibiotics used. They were all resistant to colistin sulphate. Eight of the organisms were resistant to three antibiotics, namely, amikacin, colistin sulphate, and gentamicin, except for NAS B, which was resistant to chloramphenicol and amoxicillin-clavulanic acid rather than amikacin and gentamicin. Three bacteria were resistant to only two drugs, while one was resistant to only one antibiotic. Ten of the organisms were resistant to either of the macrolide antibiotics (amikacin and gentamicin) included, while seven were resistant to both.

Table 4. Antibiogram results showing resistant strains and the total number of resistances per strain (n = 10 antibiotics/combinations).

Species	Strains	Resistance Pattern	No. of Resistance
S. aureus	STA1	AK, CT, CN	3
	STA2	AK, CT, CN	3
	STA3	CT, CN	2
	STA4	AK, CT	2
	STA5	AK, CT, CN	3
	STA6	AK, CT, CN	3
	STA7	AK, CT, CN	3
	STA8	AK, CT, CN	3
	ATCC (29213) (*S. aureus*)	CT	1
NAS	NAS A	CT, CN	2
	NAS B	CT, C, AMC	3
	NAS C	AK, CT, CN	3
	NAS D	CT	1

AK = Amikacin, CT = Colistin sulphate, CN = Gentamicin, C = Chloramphenicol, AMC = Amoxicillin-Clavulanic acid.

3.4. Thin-Layer Chromatography and Bioautography

Among the three solvent systems employed for eluting the thin-layer chromatography (TLC) plates, the non-polar solvent system, benzene: ethanol: ammonium hydroxide (BEA), exhibited the most efficient separation of compounds prior to spraying with organisms (Figures 1 and 2). An ATCC strain of *S. aureus*, one *S. aureus* strain (STA4) and one NAS strain (NAS D), were selected based on their sensitivity to the selected plant extracts. The chromatogram segments indicating white regions (marked with black ink) encircled by pinkish regions reveal the location of the active component in each extract (Figures 3–8). For some of the tested extracts against the ATCC strain, such as acetone extracts of *A. venosum* (AA), *E. caffra* (EA), and *S. lancea* (RA), multiple active compounds were observed, while for ethanol extracts of *A. venosum* (AB), *E. caffra* (EB), and *S. lancea* (RA), the separation of active compounds was not clear. Against the NAS D strain, each of the extracts showed a few separate clear zones, while against the STA4 strain, the clear zones were mostly at the origin of the TLC plate, suggesting that the active compounds were not separated by the solvent system used and were likely to be more polar. In Figures 1 and 2, the plates were sprayed with vanillin after the TLC assay. The purpose of staining the separated compounds is to distinguish the different constituents present in the extracts. The plates exhibited distinct zones of separation for each of the compounds found in the extracts. This work will serve as a guide during further investigations to determine the potential active constituents of the plant extracts.

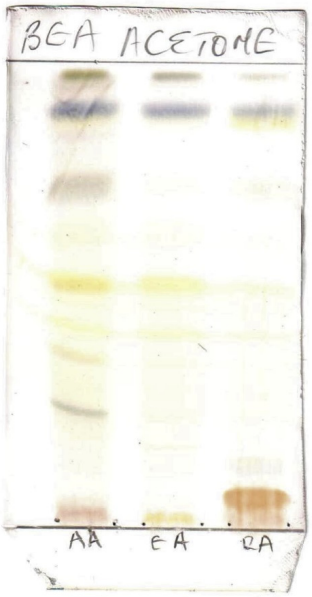

Figure 1. Chromatogram of acetone extracts. Note: AA, EA, and RA represent acetone extracts of *Antidesma venosum*, *Erythrina caffra*, and *Searsia lancea* leaves, respectively.

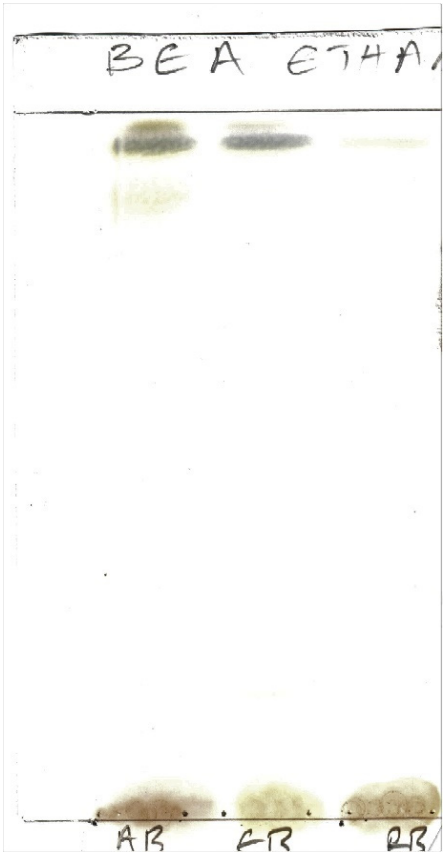

Figure 2. Chromatogram of ethanol extracts. Note: AB, EB, and RB represent ethanol extracts of *Antidesma venosum*, *Erythrina caffra*, and *Searsia lancea* leaves, respectively.

Figure 3. Acetone extracts + ATCC 29213.

Figure 4. Ethanol extracts + ATCC 29213.

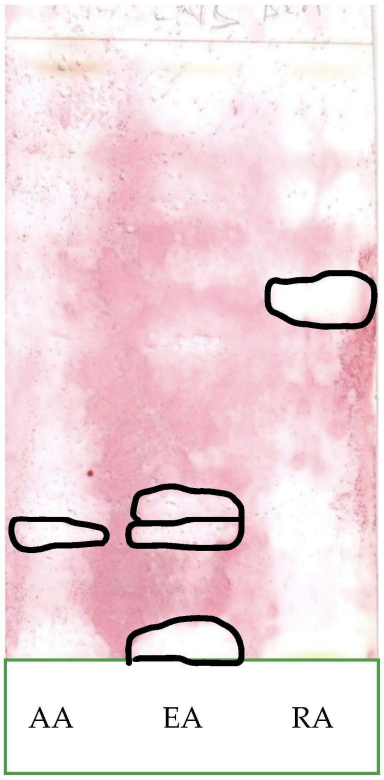

Figure 5. Acetone extracts + NAS D.

Figure 6. Ethanol extracts + NAS D.

Figure 7. Acetone extracts + STA4.

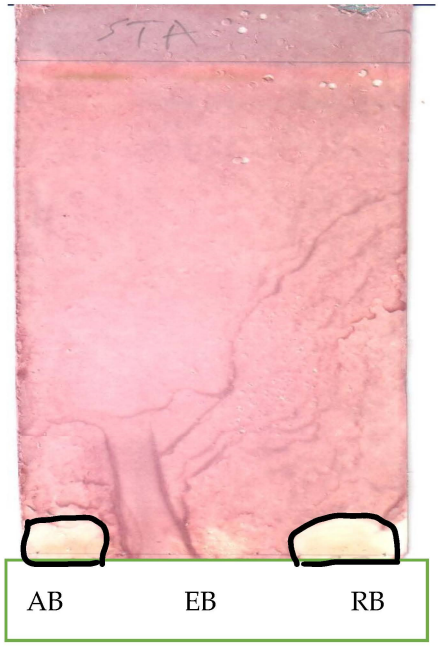

Figure 8. Ethanol extracts + STA4.

3.5. Antibacterial Activity (Minimum Inhibitory Concentration)

In this study, the activity of a plant extract was regarded as good if the MIC was less than 0.1 mg/mL, moderate if the MIC was between 0.1 and 0.63 mg/mL, and weak if the MIC was greater than 0.63 mg/mL [31].

Table 5 illustrates that the acetone extract of *S. lancea* had significant activity against all organisms with MIC values ranging from 0.01 to 0.07 mg/mL. The MIC value of 0.01 mg/mL against STA3 was the best-observed value for *S. lancea*, while the lowest MIC value of 1.41 mg/mL for *T. emetica* was recorded against STA2. Apart from *S. lancea*, only two other plant extracts out of the remaining eight demonstrated MIC values less than 0.1 mg/mL against any of the bacterial strains. *I. frutescens* exhibited good activity

with MIC values of 0.05 mg/mL and 0.09 mg/mL against STA4 and STA3, respectively, while *A. venosum* displayed good activity (MIC value = 0.09 mg/mL) against STA3 only. *S. leptodictya* (a member of the same genus as *S. lancea*) showed moderate activities against all the organisms, while *E. caffra*, *P. capensis*, *Z. mucronata*, and *T. emetica* showed moderate to poor activities against all the strains of the isolates.

Table 6 illustrates that the moderate antimicrobial activity of the ethanol extract of *S. lancea* was observed against all organisms except for NAS C, against which it showed good activity with MIC = 0.08 mg/mL. The lowest MIC value of 0.08 mg/mL was obtained for *S. lancea* against NAS C and *E. caffra* against STA4, whereas the highest MIC value of 0.94 mg/mL was recorded for *S. leptodictya* against STA7 and *T. emetica* against STA3, STA6, and NAS B. *E. caffra* demonstrated good activity (MIC value = 0.08 mg/mL) against STA4, but it showed only moderate activity against the rest of the isolates. Similar to its acetone counterpart, the ethanol extract of *S. leptodictya* displayed moderate activity against all the organisms except for STA7, against which it exhibited poor activity (0.94 mg/mL). *A. venosum* showed good to moderate activities, while *P. capensis*, *Z. mucronata*, and *T. emetica* demonstrated moderate to poor activities against all strains.

Table 7 represents the MIC range of the plant extracts against the various groups of bacteria, highlighting that several plant extracts had promising average MIC values against several bacterial strains.

3.6. Total Antibacterial Activity

The potency of plant extracts can be measured using total antibacterial activity (TAA), which takes into account both the extract yield in mg/g and the MIC in mg/mL against the target organisms [32]. To calculate the TAA, the yield of each extract was divided by the mean MIC value of each extract against the group of bacterial isolates. The resulting TAA indicates the dilution volume (in mL), at which the extract obtained from one gram of plant material can still inhibit the growth of bacteria. In this study, the acetone extract of *S. lancea* showed the highest mean TAA of 2385.87 mL/g against *S. haemolyticus*, 1964.02 mL/g against *S. aureus*, and 1291.18 mL/g against *S. chromogenes* (as shown in Table 8). The ethanol extract of *S. lancea* followed closely behind in terms of TAA. TAA values can guide the selection of plant extracts for further compound isolation and bioprospecting.

3.7. Cytotoxicity and Selectivity Indices of Plant Extracts

Two (*A. venosum* and *S. lancea*) of the three plants whose acetone extracts showed MIC values below 0.1 mg/mL and one plant (*E. caffra*) whose ethanol extract showed 0.1 mg/mL (apart from *S. lancea*), against any of the bacteria, were chosen for cytotoxic evaluation. As shown in Table 9, all the plant extracts tested had LC_{50} values higher than the cut-off cytotoxic concentration of 0.02 mg/mL [31]. Both extracts of *E. caffra* had the lowest cytotoxicity (LC_{50} > 1 mg/mL) followed by the ethanol extract *S. lancea* (0.79 mg/mL) and *A. venosum* (0.43 mg/mL). Of all the samples tested, the acetone extract of *A. venosum* was the most cytotoxic (0.08 mg/mL).

Table 5. Minimum inhibitory concentration (mg/mL) of the acetone crude extracts of selected plants against different staphylococcal bacteria isolated from mastitic cattle.

	Mean ± SEM												
Plants	**STA1**	**STA2**	**STA3**	**STA4**	**STA5**	**STA6**	**STA7**	**STA8**	**NAS A**	**NAS B**	**NAS C**	**NAS D**	**ATCC**
Pleurostylia capensis	0.23 ± 0.09	0.31 ± 0.00	0.31 ± 0.00	0.23 ± 0.09	0.31 ± 0.00	0.23 ± 0.09	0.18 ± 0.15	0.39 ± 0.26	0.23 ± 0.09	0.23 ± 0.09	0.23 ± 0.09	0.73 ± 0.43	0.47 ± 0.17
Antidesma venosum	0.12 ± 0.04	0.14 ± 0.03	0.09 ± 0.07	0.10 ± 0.06	0.12 ± 0.04	0.27 ± 0.30	0.20 ± 0.13	0.64 ± 0.66	0.20 ± 0.13	0.16 ± 0.00	0.10 ± 0.06	0.18 ± 0.15	0.12 ± 0.04
Searsia leptodictya	0.31 ± 0.00	0.20 ± 0.13	0.23 ± 0.09	0.12 ± 0.04	0.16 ± 0.00	0.29 ± 0.06	0.23 ± 0.09	0.31 ± 0.00	0.23 ± 0.09	0.23 ± 0.09	0.12 ± 0.04	0.29 ± 0.06	0.16 ± 0.00
Searsia lancea	0.05 ± 0.02	0.04 ± 0.01	0.01 ± 0.00	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.05 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.07 ± 0.02
Indigofera frutescens	0.16 ± 0.00	0.37 ± 0.13	0.09 ± 0.03	0.05 ± 0.03	0.42 ± 0.16	0.10 ± 0.04	0.10 ± 0.06	0.12 ± 0.04	0.10 ± 0.04	0.14 ± 0.03	0.12 ± 0.04	0.12 ± 0.04	0.16 ± 0.00
Erythrina caffra	0.47 ± 0.17	0.47 ± 0.17	0.31 ± 0.00	0.31 ± 0.00	0.63 ± 0.00	0.39 ± 0.26	0.42 ± 0.16	0.37 ± 0.13	0.63 ± 0.00	0.39 ± 0.26	0.73 ± 0.57	0.31 ± 0.00	0.16 ± 0.00
Elaeodendron croceum	0.63 ± 0.00	0.47 ± 0.17	0.31 ± 0.00	0.47 ± 0.17	0.73 ± 0.26	0.47 ± 0.17	0.31 ± 0.00	0.63 ± 0.00	0.31 ± 0.00	0.18 ± 0.06	0.12 ± 0.04	0.39 ± 0.26	0.47 ± 0.17
Ziziphus mucronata	0.78 ± 0.51	0.94 ± 0.34	0.23 ± 0.09	0.47 ± 0.17	0.47 ± 0.17	0.31 ± 0.00	0.26 ± 0.21	0.08 ± 0.00	0.47 ± 0.17	0.63 ± 0.00	0.78 ± 0.51	0.47 ± 0.17	0.63 ± 0.00
Trichilia emetica	0.47 ± 0.17	1.41 ± 1.20	1.29 ± 1.33	0.23 ± 0.09	0.23 ± 0.09	0.63 ± 0.00	0.35 ± 0.30	0.20 ± 0.13	0.21 ± 0.12	0.39 ± 0.26	0.39 ± 0.26	0.31 ± 0.00	0.31 ± 0.00
Gentamicin (µg/mL)	14.6	4.7	0.8	0.2	9.1	>25.0	>25.0	>25.0	>25.0	4.0	0.7	>25.0	<0.2

STA = *Staphylococcus aureus*, NAS = Non-aureus staphylococci, ATCC = American type culture collection, MIC = Minimum inhibitory concentration, SEM = Standard error of the mean.

Table 6. Minimum inhibitory concentration (mg/mL) of the ethanol crude extracts of selected plants against different strains of bacteria.

	Mean MIC (mg/mL) ± SEM												
Plants	**STA1**	**STA2**	**STA3**	**STA4**	**STA5**	**STA6**	**STA7**	**STA8**	**NAS A**	**NAS B**	**NAS C**	**NAS D**	**ATCC**
Pleurostylia capensis	0.78 ± 0.51	0.63 ± 0.00	0.57 ± 0.13	0.42 ± 0.16	0.63 ± 0.00	0.63 ± 0.00	0.63 ± 0.00	0.47 ± 0.17	0.63 ± 0.00	0.57 ± 0.13	0.31 ± 0.00	0.57 ± 0.13	0.94 ± 0.34
Antidesma venosum	0.47 ± 0.17	0.16 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	0.47 ± 0.17	0.31 ± 0.00	0.31 ± 0.00	0.31 ± 0.00	0.34 ± 0.15	0.31 ± 0.00	0.16 ± 0.00	0.31 ± 0.00	0.31 ± 0.00
Searsia leptodictya	0.63 ± 0.00	0.63 ± 0.00	0.57 ± 0.13	0.47 ± 0.17	0.63 ± 0.00	0.63 ± 0.00	0.94 ± 0.34	0.31 ± 0.00	0.47 ± 0.17	0.31 ± 0.00	0.31 ± 0.00	0.63 ± 0.00	0.68 ± 0.46
Searsia lancea	0.20 ± 0.13	0.13 ± 0.04	0.13 ± 0.04	0.18 ± 0.06	0.16 ± 0.12	0.14 ± 0.09	0.13 ± 0.04	0.13 ± 0.04	0.14 ± 0.03	0.14 ± 0.03	0.08 ± 0.00	0.18 ± 0.06	0.16 ± 0.00
Indigofera frutescens	0.47 ± 0.17	0.31 ± 0.00	0.52 ± 0.16	0.63 ± 0.00	0.63 ± 0.00	0.52 ± 0.16	0.47 ± 0.17	0.37 ± 0.13	0.31 ± 0.00	0.47 ± 0.17	0.26 ± 0.08	0.63 ± 0.00	0.63 ± 0.00
Erythrina caffra	0.12 ± 0.04	0.12 ± 0.04	0.12 ± 0.04	0.08 ± 0.00	0.12 ± 0.04	0.12 ± 0.04	0.12 ± 0.04	0.47 ± 0.17	0.16 ± 0.00	0.26 ± 0.08	0.31 ± 0.00	0.16 ± 0.00	0.31 ± 0.00
Elaeodendron croceum	0.44 ± 0.21	0.31 ± 0.00	0.31 ± 0.00	0.57 ± 0.13	0.31 ± 0.00	0.47 ± 0.17	0.47 ± 0.17	0.42 ± 0.16	0.37 ± 0.13	0.31 ± 0.00	0.23 ± 0.09	0.31 ± 0.00	0.31 ± 0.00
Ziziphus mucronata	0.47 ± 0.17	0.78 ± 0.51	0.42 ± 0.16	0.63 ± 0.00	0.78 ± 0.51	0.73 ± 0.26	1.15 ± 0.81	0.63 ± 0.00	0.73 ± 0.26	0.73 ± 0.26	0.63 ± 0.00	0.47 ± 0.17	0.83 ± 0.47
Trichilia emetica	0.52 ± 0.16	0.63 ± 0.00	0.94 ± 0.34	0.73 ± 0.26	0.63 ± 0.00	0.94 ± 0.34	0.63 ± 0.00	0.63 ± 0.00	0.57 ± 0.13	0.94 ± 0.34	0.47 ± 0.17	0.63 ± 0.00	0.83 ± 0.32
Gentamicin (µg/mL)	14.6	4.7	20.8	0.2	9.1	>25.0	>25.0	>25.0	>25.0	4.0	0.7	>25.0	<0.2

STA = *Staphylococcus aureus*, NAS = Non-aureus staphylococci, ATCC = American type culture collection, MIC = Minimum inhibitory concentration, SEM = Standard error of the mean.

Table 7. Antibacterial activity result showing the MIC range of both extracts across the groups of the bacteria strains.

MIC Range and Average MIC of the Extracts (mg/mL) ± SEM										
Plants	**MIC Range**		**STA Strains**		**NAS Strains**		**STA and NAS Strains ***		**ATCC Strain**	
Solvents	**Acetone**	**Ethanol**	**Acetone**	**Ethanol**	**Acetone**	**Ethanol**	**Acetone**	**Ethanol**	**Acetone**	**Ethanol**
Pleurostylia capensis	0.18–0.73	0.31–0.78	0.25 ± 0.06	0.59 ± 0.11	0.38 ± 0.24	0.52 ± 0.14	0.29 ± 0.15	0.57 ± 0.12	0.47 ± 0.17	0.94 ± 0.34
Antidesma venosum	0.09–0.64	0.16–0.47	0.15 ± 0.06	0.33 ± 0.22	0.14 ± 0.05	0.28 ± 0.08	**0.15 ± 0.06**	**0.31 ± 0.09**	0.12 ± 0.04	0.31 ± 0.00
Searsia leptodictya	0.12–0.31	0.31–0.94	0.22 ± 0.06	0.60 ± 0.33	0.22 ± 0.07	0.43 ± 0.15	**0.22 ± 0.06**	0.54 ± 0.18	0.16 ± 0.00	0.68 ± 0.46
Searsia lancea	0.01–0.05	0.08–0.20	0.03 ± 0.01	0.15 ± 0.44	0.04 ± 0.02	0.14 ± 0.04	**0.03 ± 0.01**	**0.15 ± 0.03**	0.07 ± 0.02	0.16 ± 0.00
Indigofera frutescens	0.05–0.37	0.26–0.63	0.17 ± 0.14	0.49 ± 0.55	0.11 ± 0.01	0.42 ± 0.16	**0.15 ± 0.12**	0.46 ± 0.13	0.16 ± 0.00	0.63 ± 0.00
Erythrina caffra	0.31–0.73	0.08–0.47	0.43 ± 0.10	0.16 ± 0.66	0.50 ± 0.21	0.22 ± 0.08	0.45 ± 0.14	**0.18 ± 0.11**	0.16 ± 0.00	0.31 ± 0.00
Elaeodendron croceum	0.12–0.73	0.23–0.47	0.46 ± 0.15	0.41 ± 0.77	0.28 ± 0.12	0.31 ± 0.05	0.40 ± 0.16	0.38 ± 0.10	0.47 ± 0.17	0.31 ± 0.00
Ziziphus mucronata	0.23–0.94	0.42–1.15	0.47 ± 0.26	0.70 ± 0.88	0.49 ± 0.22	0.64 ± 0.12	0.47 ± 0.24	0.68 ± 0.19	0.63 ± 0.00	0.83 ± 0.47
Trichilia emetica	0.21–1.41	0.47–0.94	0.56 ± 0.50	0.70 ± 0.99	0.55 ± 0.50	0.65 ± 0.20	0.56 ± 0.47	0.69 ± 0.16	0.31 ± 0.00	0.83 ± 0.32

* Bold indicates the best three average MIC values against both staphylococcal groups. SEM = Standard error of the mean, STA = *Staphylococcus aureus*, NAS = Non-aureus staphylococci, ATCC = American type culture collection, MIC = Minimum inhibitory concentration.

Table 8. Yield of acetone and ethanol extracts of the plants, total activity, and mean MIC values of the *Staphylococcus aureus*, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus* isolates and ATCC strain of *Staphylococcus aureus*.

Plants	**% Yield**		***S. aureus* Isolates**				***S. chromogenes* Isolates**				***S. haemolyticus* Isolates**				***S. aureus* ATCC Strain**			
			Mean MIC (mg/mL)		**Total Activity**		**Mean MIC (mg/mL)**		**Total Activity**		**Mean MIC (mg/mL)**		**Total Activity**		**Mean MIC (mg/mL)**		**Total Activity**	
	Ace	**Eth**	**Ace**	**Eth**	**Ace**	**Eth**	**Ace**	**Eth**	**Ace**	**Eth**	**Ace**	**Eth**	**Ace**	**Eth**	**Ace**	**Eth**	**Ace**	**Eth**
Pleurostylia capensis	51.10	67.98	0.25 ± 0.06	0.59 ± 0.11	204.96	114.71	0.40 ± 0.29	0.50 ± 0.17	127.93	134.96	0.23 ± 0.09	0.57 ± 0.13	217.91	118.63	0.47 ± 0.17	0.94 ± 0.34	108.96	72.51
Antidesma venosum	19.20	32.75	0.15 ± 0.06	0.33 ± 0.22	125.49	98.53	0.16 ± 0.05	0.27 ± 0.17	122.81	121.62	0.10 ± 0.06	0.31 ± 0.00	123.08	104.63	0.12 ± 0.04	0.31 ± 0.00	164.10	104.63
Searsia leptodictya	19.75	39.95	0.22 ± 0.06	0.60 ± 0.33	89.17	66.69	0.21 ± 0.09	0.47 ± 0.17	92.82	85.18	0.12 ± 0.04	0.31 ± 0.00	84.22	127.64	0.16 ± 0.00	0.68 ± 0.46	126.60	58.98
Searsia lancea	54.88	107.53	0.03 ± 0.01	0.15 ± 0.44	1964.02	714.65	0.04 ± 0.01	0.13 ± 0.17	1291.18	800.10	0.04 ± 0.01	0.14 ± 0.03	2385.87	751.92	0.07 ± 0.02	0.16 ± 0.00	767.48	689.26
Indigofera frutescens	16.78	68.73	0.17 ± 0.14	0.49 ± 0.55	97.46	140.69	0.11 ± 0.01	0.40 ± 0.17	148.89	172.00	0.05 ± 0.03	0.47 ± 0.17	117.31	146.54	0.16 ± 0.00	0.63 ± 0.00	107.53	109.96
Erythrina caffra	53.48	26.83	0.43 ± 0.10	0.16 ± 0.66	125.33	171.82	0.56 ± 0.22	0.21 ± 0.17	96.23	128.76	0.31 ± 0.00	0.26 ± 0.08	136.94	102.91	0.16 ± 0.00	0.31 ± 0.00	342.79	85.70
Elaeodendron croceum	49.60	81.18	0.46 ± 0.15	0.41 ± 0.77	107.24	196.20	0.27 ± 0.14	0.30 ± 0.17	181.35	266.88	0.47 ± 0.17	0.31 ± 0.00	272.40	259.35	0.47 ± 0.17	0.31 ± 0.00	105.76	259.35
Ziziphus mucronata	27.98	60.38	0.47 ± 0.26	0.70 ± 0.88	60.07	86.65	0.57 ± 0.18	0.61 ± 0.17	48.81	99.35	0.47 ± 0.17	0.73 ± 0.26	44.76	82.80	0.63 ± 0.00	0.83 ± 0.47	44.76	72.44
Trichilia emetica	27.90	69.90	0.56 ± 0.50	0.70 ± 0.99	49.68	99.41	0.30 ± 0.09	0.56 ± 0.17	91.78	125.79	0.23 ± 0.09	0.94 ± 0.34	71.45	74.56	0.31 ± 0.00	0.83 ± 0.32	89.14	83.88

ATCC = American type culture collection, MIC = Minimum inhibitory concentration, mg/mL = milligram per millimeter, Ace = Acetone, Eth = Ethanol.

Table 9. Cytotoxicity against Vero cells LC_{50} (mg/mL) and selectivity indices of the six selected crude extracts.

			Selectivity Index of Selected Plant Extracts													
Plants	**Extractant**	**LC_{50} (mg/mL)**	**Mean SI**	**STA1**	**STA2**	**STA3**	**STA4**	**STA5**	**STA6**	**STA7**	**STA8**	**NAS A**	**NAS B**	**NAS C**	**NAS D**	**STA ATCC**
Antidesma venosum	Ace	0.08 ± 0.00	0.65 ± 0.15	0.68	0.56	0.91	0.82	0.68	0.30	0.68	0.68	0.68	0.68	0.68	0.45	0.67
Antidesma venosum	Eth	0.43 ± 0.16	1.24 ± 0.51	0.92	2.76	1.37	1.37	0.92	1.37	0.92	0.92	0.92	0.92	0.92	1.37	1.39
Searsia lancea	Ace	0.15 ± 0.02	4.69 ± 0.89	2.88	4.13	10.27	4.13	8.11	10.23	2.88	2.88	2.88	2.88	2.88	4.71	2.14
Searsia lancea	Eth	0.79 ± 0.09	4.64 ± 0.78	4.04	6.08	6.08	4.34	4.85	5.52	4.04	4.04	4.04	4.04	4.04	4.34	4.94
Erythrina caffra	Ace	>1	2.69 ± 1.18	2.13	2.13	3.19	3.19	1.60	2.56	2.13	2.13	2.13	2.13	2.13	3.19	6.25
Erythrina caffra	Eth	>1	8.30 ± 2.05	8.55	8.55	8.55	12.82	8.55	8.55	8.55	8.55	8.55	8.55	8.55	6.41	3.23

STA = *Staphylococcus aureus*, NAS = Non-aureus staphylococci, Ace = Acetone, Eth = Ethanol, ATCC = American type culture collection, LC = Lethal concentration.

In relation to the MICs of the extracts against each of the organisms tested, the ethanol extract of *E. caffra* had the best mean selectivity index (SI) (8.30 ± 2.05) against all the pathogens (Table 9), while the acetone extract of *A. venosum* had a poor mean SI (0.65 ± 0.15).

4. Discussion

4.1. Plant Yield

Generally, the ethanol extracts of all the plants yielded more than the acetone extracts, except in the case of *Erythrina caffra*, where the acetone extract yielded more than the ethanol extract. It is well established that ethanol has a high capacity to permeate cell membranes, allowing for the extraction of greater amounts of intracellular components compared to solvents with lower polarity [33]. Therefore, the extract yield plays a significant role in determining the total activity of plant extracts, which is crucial in comparing them for bioprospecting purposes [29].

4.2. Antibiogram

Worthy of note is the fact that gentamicin at concentrations ranging between 0.2 and 25.0 µg/mL was used as the positive control in the quantitative antibacterial assay, and against most of the organisms, gentamicin had relatively poor activity (MIC above 9.1 µg/mL) as shown in Tables 5 and 6. Since the concentration of gentamicin in the antibiogram disc is 10 µg, there was a correlation between the resistance patterns shown by the organisms in the MIC and the antibiogram assays.

4.3. Antibacterial Activity

To the best of our knowledge, the plants utilized in this research have not been previously assessed against *Staphylococcus* spp. derived from mastitis. However, various studies have shown the antibacterial activities of extracts from these plants against *Staphylococcus* spp. from different sources and other plants against *Staphylococcus* spp. isolated from bovine mastitis cases. For instance, the essential oil extracted from *S. lancea* has been reported to possess antibacterial activity against *S. aureus*, with a MIC value of 0.01 mg/mL [34], while the 70% methanol extract of the plant's leaf showed a MIC of 0.06 mg/mL [35]. Considering the MIC values obtained with the acetone extract of *S. lancea* against isolates of *S. aureus* in this work, together with these examples, leaf extracts of *S. lancea* prepared using different solvents have antibacterial activities against *S. aureus*.

The result obtained in this investigation using the acetone extract of *S. leptodictya* against the ATCC strain of *S. aureus* corresponds with another report of the same MIC value of 0.16 mg/mL against the same strain of *S. aureus* as was found in this study [36]. Meanwhile, another study reported MIC of 0.60 mg/mL with the acetone leaf extract of *T. emetica* against *S. aureus* [37], which is higher than the MIC values obtained against six of the *S. aureus* strains used in this study with the same extract.

A striking observation from Tables 5 and 6 is that only the acetone extract of *S. lancea* showed good activity against all the strains of *S. aureus* and NAS. Neither the ethanol extract of *S. lancea* nor both (acetone and ethanol) extracts of other plants had good activity against any strain of NAS. The results also suggest that the *S. aureus* strains were more susceptible to the extracts than the NAS strains. A similar pattern of susceptibility was reported by other researchers [38], although they used a disc diffusion method. Significant antibacterial properties against mastitis-causing bacteria were observed with all the extracts obtained from *Liquidambar orientalis* leaf using three different solvents (acetone, methanol, and ethanol). However, the antibacterial activity of these extracts was significantly poorer against NAS species than against *S. aureus*. The acetone extracts showed the highest antibacterial activity (with a 12 mm inhibition zone diameter) against *S. aureus* 17, while the lowest antibacterial activity (with a 7 mm inhibition zone diameter) of acetone extracts was found against NAS-36 [38]. Another study reported varying susceptibility patterns regarding the essential oils obtained from selected plants [38]. They reported that some isolates of NAS (*S.*

epidermidis and *S. xylosus*) (MIC = 0.156%) appeared to be less susceptible than some isolates of *S. aureus* to the essential oil of *Pelargonium graveolens* (MIC = 0.078%). Furthermore, they also reported some isolates of both *S. aureus* and NAS that were susceptible at the same concentration to essential oils of other plants, such as *Juniperus virginiana* (MIC = 0.010%), *Leptospermum scoparium* (MIC = 0.005%), *Pogostemom cablin* (MIC = 0.010%), and *Thymus vulgaris* (MIC = 0.010%) [39]. In another study, it was reported that the acetone leaf extract of *Acacia nilotica* showed higher antibacterial activity against *S. epidermidis* and *S. chromogenes* (MIC = 0.156 mg/mL) than *S. aureus* (MIC = 0.625 mg/mL) [40]. This same pattern was also reported [39], claiming that some isolates of NAS (MIC = 0.020%) appeared to be more susceptible than some isolates of *S. aureus* (MIC = 0.156%) to the essential oil of *Cinnamomum cassia*. The acetone leaf extract of *Aloe arborescens* showed a similar MIC (>2.5 mg/mL) against *S. aureus* and *S. chromogenes*, and a lower MIC (2.5 mg/mL) against *S. epidermidis* [40]. Further research needs to be conducted to unravel the reason for these patterns. Knowing that the NAS and STA strains are Gram-positive organisms, this observed pattern may not be due to the cell wall infrastructure of both groups of bacteria. This variation in susceptibility patterns of *S. aureus* and NAS isolates may likely be due to the differences in the antibacterial compounds in the different extracts or samples. It might be important to isolate and investigate these compounds and co-formulate them to produce a broad-spectrum product that can be used in the management of bovine mastitis caused by these organisms.

According to Table 7, NAS strains were more susceptible to the extracts over a shorter MIC range than *S. aureus* strains, which are susceptible over a wider MIC range. This is similar also to a previous study which reported geranium oil to have a wider MIC range (0.078–1.25%) for *S. aureus* and a narrower MIC range (0.156–1.25%) for NAS [39]. Knowing that NAS is a group of species of *Staphylococcus*, it would be expected that their susceptibility may vary more widely than the strains of *S. aureus* which are the same species. To observe the pattern in the opposite direction suggests that the species (or strains) of NAS used in this study have a more similar structural, biochemical, and/or physiological response to the extracts than the strains of *S. aureus* used in the study. This may suggest the possibility of extensive genetic variations among the strains of *S. aureus* used in this study. This calls for biomolecular investigations to understand the extent of the genetic variations among strains of the same species that are responsible for their varying susceptibility to the same extract.

The average MIC of the extracts against all bacteria (Table 7; column "STA & NAS strains") showed that only the acetone extract of *S. lancea* showed excellent antibacterial activity (mean MIC = 0.03 mg/mL). The acetone extracts of the other plants showed only moderate activity, while the ethanol extracts of all the plants showed moderate to poor activity. The average MIC values of all the acetone extracts of each of the plants were lower than those of their ethanol counterparts, except for *E. caffra*. The average MIC value of the ethanol extract of *E. caffra* (mean MIC = 0.18 mg/mL) for each of the bacteria appears to be less than half of the average MIC value of its acetone extract (mean MIC = 0.45 mg/mL). This is an interesting observation since, though acetone is mostly the preferred extractant due to the observed better antibacterial activity, ethanol remains the suitable solvent due to the potential to commercialize the findings of this study. This is due to the fact that ethanol is not as flammable and less dangerous to work with in large quantities compared to acetone. Moreover, ethanol is able to effectively permeate cell membranes, which facilitates the extraction of a wide range of polar and non-polar components from inside the cell, permitting the extraction of higher levels of polar and non-polar endo-cellular components [33]. Therefore, the ethanol extract of *E. caffra* should be further investigated. The observation of better activities with the acetone extract of the other plants is consistent with various studies, which have suggested acetone to be the preferred solvent of extraction for antimicrobial investigation of plants since acetone can extract compounds of a wider range of polarity [18,19].

Against the ATCC strain of *S. aureus*, only the acetone extracts of *S. lancea* had good activity (MIC = 0.07 mg/mL). Both ethanol and acetone extracts of the other plants showed moderate activities, except for the ethanol extracts of *P. capensis* (MIC = 0.94 mg/mL), *Z. mucronata* (MIC = 0.83 mg/mL), and *T. emetica* (MIC = 0.83 mg/mL) which showed poor activities. It generally appears that the isolates were more susceptible to the extracts than the ATCC strain, which is an interesting result, while the ATCC strain was more susceptible to gentamicin (positive control) than the isolates (Tables 5 and 6).

The antibacterial investigation suggests the potential usefulness of the extract of *S. lancea* as an antibacterial agent with a broad spectrum of activity against staphylococci implicated in causing bovine mastitis.

4.4. Cytotoxicity

When the LC_{50} is 0.02 mg/mL or lower, a plant extract is classified as cytotoxic [32]. Based on the above definition, all the tested plant extracts in this study were relatively non-cytotoxic as the lowest value obtained was 0.08 mg/mL (Table 9), which is above the cut-off value.

However, another study reported the LC_{50} of acetone and ethanol extracts of *S. lancea* extracts to be below 0.05 mg/mL, which is lower compared to the values obtained in this work [41]. They observed that the acetone and ethanol extracts of *S. lancea* exhibited moderate toxicity toward Vero cells at concentrations of 0.25 and 0.05 mg/mL, respectively. In contrast, the ethyl acetate extract of *S. lancea* showed no toxicity at the same concentrations. Therefore, they hypothesized that the presence of alkaloids and saponins in *S. lancea* could be responsible for its observed toxicity [41]. LC_{50} values of 0.025, 0.022, and 0.051 mg/mL against Vero cells by other species of the *Searsia* genus, such as *S. leptodictya*, *S. pendulina*, and *S. pentheri*, respectively have been reported [42]. Moreover, the cytotoxic activity of water extracts of *S. lancea* leaf material has been reported against brine shrimp with LC_{50} value of 0.6 mg/mL [43].

According to reports, the n-hexane and ethyl acetate extracts derived from the root bark of *E. caffra* demonstrated cytotoxic effects on human cervical carcinoma cells at concentrations of 0.11 mg/mL and 0.06 mg/mL, respectively [44]. Another study found that the ethanol extract of the stem bark of *A. venosum* exhibited greater toxicity (LC_{50} = 0.026–0.041 mg/mL) compared to the root extract (LC_{50} = 0.063–0.080 mg/mL) against brine shrimp [45]. However, it should be noted that in vitro cellular toxicity may not necessarily reflect in vivo toxicity due to various factors, such as gut interactions and bioavailability. Therefore, further studies involving acute and chronic animal toxicity testing are necessary to confirm the safety of these plant extracts [42]. The LC_{50} of ethanol extracts of the plants in this study were higher than those of their acetone counterparts, which suggests that the ethanol extracts are relatively less toxic compared to their acetone counterparts. The results obtained by [45] also favor ethanol extracts over acetone extracts. This is significant for potential commercialization as ethanol is preferred as a solvent for industrial use since it is less flammable and dangerous to work with in large quantities.

To determine the safety margin of a plant extract, the selectivity index (SI) is calculated using two variables: Cytotoxicity (mg/mL) and minimum inhibitory concentration (MIC) values [46]. If the selectivity index (SI) value is greater than 1, it indicates that the plant extract is more harmful to the pathogen than to the mammalian cells tested for cytotoxicity. A higher SI value is more encouraging since it suggests that the plant extract's activity is not due to general toxicity. Consequently, the higher the SI, the greater the potential for the plant extract to be developed into a safe herbal product. The acetone extracts of *S. lancea*, which showed the most promising antibacterial activity against all the organisms tested, also had a very promising LC_{50} value of 0.15 ± 0.02, SI range of 2.14 to 10.23, and mean SI of 4.69 ± 0.89. Based on the results obtained, it can be concluded that although the extracts of all three plants tested have the potential to be developed as safe and effective herbal remedies for treating microbial infections, *S. lancea* seems to be the most promising candidate.

4.5. Limitations of the Study and Future Studies

This study aimed to evaluate the antibacterial efficacy and cytotoxicity of extracts of nine selected plant species. Test bacteria included drug-resistant strains of *S. aureus* and NAS isolated from clinical cases of bovine mastitis. The cytotoxic effects of extracts of three plants selected according to their promising antibacterial activities were also determined. In vitro studies have limitations insofar as biological activity in the laboratory cannot replicate conditions in vivo; therefore, biological activity and toxicity studies need to be conducted, preferably in dairy cows. Future work will involve developing and testing formulations containing active extracts for topical application to udders of dairy cows in order to assess their ability to prevent and treat mastitis. Additionally, isolation and identification of bioactive compounds from the most antibacterially active and least cytotoxic plant extracts will be conducted to identify potential marker compounds. Furthermore, synergistic activity of different plant extracts and their components will be explored.

5. Conclusions

The antimicrobial activities of the selected plants against *S. aureus* and NAS isolated from clinical cases of bovine mastitis are not well known, although some of the plants in this study are recognized for their activities against other microbes. However, the crude extracts of these plants exhibited good to moderate antibacterial activity against drug-resistant staphylococcal isolates, and it generally appears that the isolates were more susceptible to the extracts than the ATCC strain. Among the plants tested, *S. lancea* exhibited the most potent antibacterial activity, suggesting its potential usefulness as a broad-spectrum antibacterial agent against staphylococci associated with bovine mastitis, at relatively non-cytotoxic concentrations. The ethanol extract of *E. caffra* appears to be active against the organisms at a concentration less than half of its acetone counterpart. This favors the preference for ethanol in industrial scale extraction of plant materials. Moreover, the ethanol extracts of *A. venosum*, *E. caffra*, and *S. lancea* demonstrated low toxicity to mammalian cells, making them attractive candidates for possible development into herbal products or for isolating novel pure compounds that can serve as templates for new antimicrobial drugs, which can be viable alternatives for managing bovine mastitis. Future studies will investigate the synergistic antibacterial activity of these extracts and their constituents, leading to the development of sustainable, polyherbal products useful in managing bovine mastitis.

Author Contributions: Conceptualization, A.O.A., I.M.F., I.-M.P. and L.J.M.; methodology, A.O.A. and I.M.F.; formal analysis, A.O.A. and I.M.F.; investigation, A.O.A. and I.M.F.; resources, I.-M.P. and L.J.M.; data curation, A.O.A. and I.M.F.; writing—original draft preparation, A.O.A.; writing—review and editing, I.M.F., I.-M.P. and L.J.M.; supervision, I.-M.P. and L.J.M.; project administration, A.O.A.; funding acquisition, L.J.M. All authors have read and agreed to the published version of the manuscript.

Funding: The bacterial identification is based on the research supported in part by the National Research Foundation (NRF) of South Africa (Grant specific unique reference number (UID) 74426). Lise Korsten, Zama Zulu, and the staff of the University of Pretoria's Department of Plant and Soil Sciences are hereby acknowledged for their support. The National Research Foundation (Grant number 111945) provided research funding to L.J.M. The University of Pretoria is acknowledged for bursary support to A.O.A.

Institutional Review Board Statement: This study received ethical permission from the Ethics Committee, Faculty of Veterinary Science, University of Pretoria (reference number REC236-19).

Informed Consent Statement: Not applicable.

Data Availability Statement: All research data are shared in this article.

Conflicts of Interest: The authors declare no conflict of interest.

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