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Susceptibility patterns of *Escherichia coli* and streptococcal isolates from bovine mastitis cases to antibiotics and selected South African plant extracts with known antibacterial activities

Ayodele O. Akinboye[a], Rasheed O. Adeyemo[a,c], Joanne Karzis[b], Inge-Marie Petzer[b], Lyndy J. McGaw[a,*]

[a] Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, Private Bag X04, Onderstepoort 0110, South Africa
[b] Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa
[c] Department of Microbiology and Parasitology, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda,

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A B S T R A C T

Bovine mastitis, an inflammatory condition affecting the mammary glands of dairy cattle, poses a significant economic burden on the dairy industry. *Staphylococcus* species, *Streptococcus* species and *Escherichia coli* are common pathogens. Managing mastitis typically involves antibiotics, but antimicrobial resistance (AMR) is a growing concern, promoting further research exploring alternative treatments including plant extracts. This study aimed to determine the antibiotic resistance of *E. coli* and streptococcal isolates from mastitis cases, and to investigate four indigenous South African plants for their selective antibacterial activities against these pathogens. The antimicrobial susceptibility of the bacterial strains was determined using a standard disc diffusion method. Minimum inhibitory concentration (MIC) values of acetone and ethanol extracts of *Searsia lancea, Indigofera frutescens, Erythrina caffra* and *Antidesma venosum* were determined against the bacterial isolates using a serial microdilution assay, and cytotoxicity was also investigated. The results showed that 82.14% of the clinical isolates tested were resistant to at least one antimicrobial agent used, and 52.17% of the antibiotic resistant isolates were multidrug resistant. All plant extracts had antibacterial activity against all the bacterial isolates, but *S. lancea* demonstrated higher efficacy compared to other plants. The MIC values ranged from 0.01 to 2.50 mg/mL, with the lowest range obtained with the acetone extract of *S. lancea* (0.01 to 0.57 mg/mL). Furthermore, the extracts were relatively non-cytotoxic to bovine dermis and Vero cells, with the highest mean selectivity index value of 25.70 recorded with *S. lancea*. This study highlights the growing concern of AMR in livestock management, and demonstrates the promising therapeutic potential of the selected plant species, particularly *S. lancea*, in treating bovine mastitis. Further exploration of *S. lancea* is recommended to develop novel alternative or complementary formulations for mastitis management.
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1. Introduction

Bovine mastitis is an inflammatory condition that leads to pathological changes in the mammary glands of dairy cattle. It is characterized by physical, chemical and microbial changes in the udder, leading to a decrease in milk quality and quantity (Goulart & Mellata, 2022). Milk is considered a medium favourable to bacterial growth, due to its high temperature immediately after milking (37°C), richness in nutrients and pH around 6.6 to 6.8 (Kose et al., 2015). Mastitis is a complex and multifactorial disease; its occurrence depends on variables related to the animal, environment and pathogen (Radostits et al., 2007). It is mostly triggered by pathogenic microorganisms, including bacteria, fungi and viruses, breaking through the host defence mechanisms to destroy milk-producing parenchyma tissues (Schroeder, 2012). Some bacterial species that are commonly implicated in bovine mastitis include *Staphylococcus* species, *Escherichia coli* and *Streptococcus* species (Lopes et al., 2020).

The most frequently encountered mastitis-causing pathogens include *Staphylococcus aureus* (*Sta. aureus*), *Streptococcus uberis* (*S. uberis*), *Streptococcus agalactiae* (*S. agalactiae*), *Streptococcus dysgalactiae* (*S. dysgalactiae*), *E. coli* and other coliforms (Mushi et al. 2022; Vollenweider et al., 2023). Research by Kang et al. (2022) has shown that *S. uberis* and *S. dysgalactiae* accounted for 39.2% and 29.3%, respectively, of *Streptococcus* species isolated from cases of clinical mastitis in South Korea (Kang et al., 2022), while *S. agalactiae* was isolated in 33.6% of clinical mastitis cases in a study by Han et al. (2022)

* Corresponding author.
E-mail address: lyndy.mcgaw@up.ac.za (L.J. McGaw).

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from China. In another study conducted in India, the major mastitis-causing bacteria isolated from clinical cases of bovine mastitis were *Staphylococcus* spp. (46.4%) followed by mixed infections with *Staphylococcus* and *Streptococcus* (20.8%), *Streptococcus* spp. (18.4%) and *E. coli* (14.4%) (Waseem et al., 2020).

In general, clinical and subclinical forms of mastitis can be distinguished from one another (Watts, 1990). Stage one clinical mastitis can be identified by the visible presence of an abnormal secretion that may contain floccules, and this can be watery or contain blood (Hortet & Seegers, 1998; Bartlett et al., 2001). According to Hillerton et al. (1995), sudden onset clinical mastitis, also known as acute clinical mastitis, can be associated with swelling, elevated warmth, oedema and pain in the udder. In stage 3 mastitis, changes in the secretion and udder parenchyma are accompanied by systemic symptoms such as a loss of appetite, fever, dehydration or depression and in some cases the death of the cow. Subclinical mastitis is characterized by the absence of overt alterations in either the udder or the milk, despite the presence of microorganisms in the udder. Compositional alterations and an increased somatic cell count in milk frequently follow intramammary infection (Auldist & Hubble, 1998; Watts, 1990).

Bovine mastitis is a major concern in the dairy industry due to its impact on animal health and the quality of milk produced. The economic rise of the dairy market all over the world with the importance of delivering healthy and safe dairy products highlights the importance of managing milk production in a secure and sustainable manner (Garcia et al., 2019). The disease poses a significant economic burden on the primary dairy industry due to reduced milk yield, poorer milk quality treatment costs, and shortened productive life of affected animals (Ibrahim & Ghanem, 2019). It has been estimated that the overall cost of bovine mastitis to the global dairy industry is billions of dollars per year (Gomes & Henriques, 2016).

The South African dairy industry is not spared, and annual costs of mastitis per cow per year in the year 2014 were R919.96 with an average mastitis incidence of 0.9 (Man'ombe, 2014). Banga et al. (2014) estimated the economic value of dairy cows and found that for every increase by one in somatic cell score, there was a decreased profit margin of R1 143.53 per cow per annum. Such economic value can further be affected by the type of breed, revenue incentive payment system, production system, total mixed ration, pasture base or a modification of the two (Banga et al., 2014).

Management of infectious bovine mastitis typically involves the use of antibiotics and anti-inflammatory drugs (Schukken et al., 2003). However, the discovery of each new antibiotic has been followed by reports of emerging resistance against it (Carlet et al., 2014). Unfortunately, the prevalence of antimicrobial resistance (AMR) to multiple classes of antibiotics used against mastitis-causing bacteria is high in dairy cattle (Sharifi et al., 2023). Antimicrobial resistance has emerged as a threat to the current effective treatment for an ever-increasing range of microbial infections. It results in reduced efficacy of antibiotics, making treatment complicated, time consuming, costly, or sometimes even impossible (Carlet et al., 2014).

According to Pascu et al. (2022) antibiotic resistance is a growing problem in the treatment of bovine mastitis caused by streptococcal species in Romania. Permatasari et al. (2022) and Magagula et al. (2023) found that a significant proportion of isolated strains of *S. agalactiae* and *S. uberis* from mastitic milk in Indonesia and South Africa, respectively, were resistant to multiple antibiotics, including penicillins, macrolides, and tetracyclines. Another report in China confirmed that the majority of isolated *Streptococcus* spp. were resistant to at least three antimicrobials (Tian et al., 2019). Based on a sensitivity test of bacteria against various antibiotics conducted by Permatasari et al. (2022), it was observed that *S. agalactiae* isolates were resistant to ampicillin (75%) and erythromycin (50%). In another report by My et al. (2023) in Vietnam, 50 *E. coli* isolates tested were resistant to lincomycin and sulfamethoxazole, while multidrug resistant (MDR) activity prevalence was confirmed in 46% of the isolates. Subsequently, studies have reported the presence of drug resistance genes in some streptococcal isolates from mastitis (Haenni et al., 2011; Cadona et al., 2021; Han et al., 2022). These genes can be transferred from one isolate to another via horizontal gene transfer or gene exchange, and allow the resistance to spread through a population of bacteria and among different species of bacteria, leading to increasing populations of AMR strains (Vezina et al., 2022). The prevalence of AMR strains of bacteria has led to a growing interest in alternative or complementary treatments for bovine mastitis.

Research on using plant extracts as treatments for MDR bacterial infections, or as part of preventative measures, is both promising and ongoing. Although none of the purified compounds from these extracts has been commercialized yet, they demonstrate the potential of using plant extracts as alternative treatments in the management of bovine mastitis. Recent studies have indicated that a combination of various natural products, including plant extracts, honey, propolis, prebiotics, probiotics, synbiotics and postbiotics, when used alongside conventional drug therapy, holds potential as an adjuvant treatment (Machado et al., 2023). This approach has the potential to restore drug sensitivity in MDR pathogens, enhance host immunity, and ultimately improve clinical effectiveness (Machado et al., 2023). The antimicrobial activities of some plant species have been extensively researched. Examples of such studies include the work of Šukele et al. (2023) which showed that the plant extracts tested had inhibitory effects on the growth of *E. coli, S. agalactiae, S. uberis, Serratia liquefaciens, Sta. aureus*, and reference cultures of *Sta. aureus* and *E. coli*. Plants contain several phytochemicals such as flavonoids, alkaloids, tannins and terpenoids, which are known to possess antimicrobial and antioxidant properties (Waseem et al., 2023). However, empirical studies are required to evaluate the efficacy of plant extracts (Adeyemo et al., 2022).

For this study, *Antidesma venosum, Erythrina caffra, Indigofera frutescens* and *Searsia lancea* were selected, based on our previous research where these species exhibited good antibacterial activity against AMR staphylococcal isolates of mastitis origin (Akinboye et al., 2023). This study aimed to determine the antimicrobial resistance profile of *E. coli* and streptococcal isolates from clinical cases of bovine mastitis, and the antibacterial and cytotoxic activities of these indigenous South African plants against them.

2. Materials and methods

2.1. Plant material and extraction

The leaves of the plants used in this study (Table 1) were harvested from the Manie van der Schijff Botanical Garden at the University of Pretoria and the Onderstepoort campus of the University of Pretoria. Herbarium voucher specimens were prepared and deposited in the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria. Healthy leaves were harvested and placed 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. Acetone and ethanol were used for the extraction of the plant material. Twenty grams of powdered plant leaves were soaked separately in 200 ml of respective solvent with intermittent manual shaking of

Table 1
Selected plants and their herbarium accession numbers.

Plant species	Family	Accession numbers
Indigofera frutescens L.f.	Leguminosae	PRU 128111
Searsia lancea (L.f.) F.A.Barkley	Anacardiaceae	PRU 128113
Antidesma venosum E. Mey. ex. Tul.	Phyllanthaceae	PRU 128361
Erythrina caffra Thunb.	Fabaceae	PRU 128360

the bottles. After 24 h, the supernatant was collected and filtered through Whatman No. 1 filter paper into pre-weighed glass vials and concentrated by drying under a stream of cold air. This process was repeated three times on the same plant material. The dried extracts were weighed, and the yields were obtained by dividing the mass extracted by the initial mass.

2.2. Antibacterial screening

2.2.1. Bacterial isolates

Bacterial strains (Table 2) were derived from the biobank collection of the Milk Laboratory, Department of Animal Production Studies, Faculty of Veterinary Sciences, University of Pretoria and used for this study. These bacteria were isolated from milk samples of South African dairy herds during routine laboratory testing. The isolates were maintained in Tryptic Soy Broth (TSB) with 10% glycerol, frozen at -80°C.

2.2.2. Antibiotic susceptibility testing

The streptococcal strains were subjected to antimicrobial susceptibility testing against a panel of 10 products while the *E. coli* strains were subjected to a panel of 11 products. The antibiotic products investigated and concentrations of each disc added below in Table 4. The Kirby-Bauer disc diffusion method (Bauer et al., 1966) with published breakpoints was used to determine the antimicrobial susceptibility of the isolates. The results were based on the diameter of the inhibition zones and were classified as sensitive, intermediate or resistant in accordance with the clinical breakpoints established by the Clinical Laboratory and Standards Institute (Clinical and Laboratory Standards Institute (CLSI), 2020, 2022). Isolates that were resistant to at least one antibiotic product were defined as “resistant”. The characterisation of isolates as multidrug-resistant was done according to well-established criteria (Magiorakos et al., 2012).

2.2.3. Determination of minimum inhibitory concentration (MIC)

A simple twofold serial dilution microplate method was used to determine the minimum inhibitory concentration (MIC) of the plant extracts (Eloff, 1998). Bacterial cultures grown overnight in Brain Heart Infusion (BHI) broth (Sigma Aldrich, SA) were adjusted to McFarland standard 0.5, equivalent to 1.5×10^8 CFU/mL. A 100 μl aliquot of sterile distilled water was added to all the wells of a 96-well microtitre plate. The prepared extracts (10 mg/ml stock concentrations) were added to the first row of the microplate and serially diluted in a 1:1 ratio. After that, 100 μL of adjusted bacterial cultures were added to each well. The bacteria were exposed to the extracts of final concentrations ranging between 2.5 and 0.001 mg/mL. Acetone and ciprofloxacin served as negative and positive controls, respectively. The plates were then incubated at 37°C for 18–24 h. Following incubation, 40 μl (0.2 mg/ml) of iodonitrotetrazolium violet (INT) was added to each well and incubated for 1 h. The MIC was taken as the lowest extract concentration to show growth inhibition, visible in terms of a decrease in red colour generated by the conversion of the INT to a red product by actively respiring bacteria. The total activity (mL/g) of the extracts is calculated by dividing the mass in mg extracted from 1 g of plant material by the MIC in mg/mL (Eloff, 2000).

Table 2
List of organisms and their laboratory codes.

Species	Types	Laboratory Codes
Streptococcus agalactiae	Clinical isolates	SAG 1 - 7
Streptococcus dysgalactiae	Clinical isolates	SDY 1 - 7
Streptococcus uberis	Clinical isolates	SUB 1 - 7
Streptococcus uberis	ATCC	SUB ATCC 700407
Escherichia coli	Clinical isolates	ECO 1 - 7
Escherichia coli	ATCC	ECO ATCC 25922

ATCC = American Type Culture Collection

2.3. Cytotoxicity

The cytotoxicity of the plant extracts against Vero (African green monkey kidney) cells (ATCC® CCL-81™) and bovine dermis cells was determined using the 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyltetrazolium-based colorimetric (MTT) assay as described by Mosmann (1983) and modified by McGaw et al. (2007). The cells were grown in Minimal Essential Medium (MEM) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). Cells of a sub-confluent culture were harvested and centrifuged at 200 x g for 5 min and re-suspended in MEM to 5×10^4 cells/mL. Cell suspensions (100 μL; 1×10^5 cells/mL) were pipetted into each well of columns 2 to 11 of a sterile 96-well microtitre plate. The plates were incubated for 24 h at 37°C in a 5% CO_2 incubator to allow the cells to attach and reach the exponential phase of growth. One hundred μL of the extracts at differing concentrations prepared in MEM were added to the plates in quadruplicate. The microtitre plates were incubated at 37°C in a 5% CO_2 incubator for 48 h with the plant samples. Untreated cells and positive control (doxorubicin chloride, Pfizer Laboratories) were also included. After incubation, the contents of each well were aspirated, and cells were washed with phosphate-buffered saline (PBS, Whitehead Scientific) and replaced with 200 μL of fresh MEM. Then, 30 μL MTT (Sigma, stock solution of 5 mg/mL in phosphate-buffered saline (PBS)) were added to all the wells and the plates were incubated for a further 4 h at 37°C. After incubation, the medium in each well was carefully removed, without disturbing the MTT crystals in the wells. The cells were washed with PBS and the MTT formazan crystals were then dissolved by adding 50 μL of dimethyl sulfoxide (DMSO) to all the wells. The plates were shaken gently to allow the MTT solution to dissolve. The amount of MTT reduction was measured immediately 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 of absorbance (correlating to killing 50% of the cells) compared to untreated cells. The LC_{50} values were used to calculate the selectivity index (SI) by dividing the LC_{50} by the MIC values (both in mg/mL) of each organism: SI = LC_{50}/MIC.

2.4. Statistical analysis

Data were entered and collated in Microsoft Excel 365, and IBM SPSS 27 was used for data analysis using one-way analysis of variance (ANOVA) and Tukey’s post hoc test where appropriate, with a significance level of $p < 0.05$ in Table 6.

3. Results

3.1. Plant extract yield

Table 3 shows the amount of dry extracts obtained from 100 g of dried ground leaves of each of the four plants. The ethanol extract of *Searsia lancea* had the highest dry mass (15.79 g), followed by the ethanol extract of *Indigofera frutescens* with 8.41 g, while the acetone

Table 3
Percentage yield of the plant extracts from 100 g leaf material.

Plants	Extractant	Yield (g)	% Yield (g/g)
Searsia lancea	Acetone	5.82	5.82
	Ethanol	15.79	15.79
Erythrina caffra	Acetone	3.51	3.51
	Ethanol	4.77	4.77
Antidesma venosum	Acetone	1.74	1.74
	Ethanol	3.03	3.03
Indigofera frutescens	Acetone	2.97	2.97
	Ethanol	8.41	8.41

g = gram, % = percentage

Table 4
Antimicrobial resistance pattern of the test bacteria

Bacteria (Lab. Code)	P	AMP	AMC	OX	TE	CFX/K	KF	RAX	FOX	EFT	N	K	CN	PB
ECO1	-	-	S	-	S	R	R	R	R	S	R	S	I	S
ECO2	-	-	S	-	S	R	R	R	R	S	I	S	S	S
ECO3	-	-	R	-	S	R	R	I	I	R	I	I	S	R
ECO4	-	-	S	-	S	R	R	R	R	S	I	I	R	S
ECO5	-	-	R	-	S	R	R	R	I	R	R	I	S	S
ECO6	-	-	R	-	S	R	R	R	R	I	R	I	S	R
ECO7	-	-	R	-	R	R	R	R	I	S	I	I	S	R
SAG1	S	S	S	R	S	S	S	S	S	S	-	-	-	-
SAG2	S	S	S	S	S	S	S	S	S	S	-	-	-	-
SAG3	S	R	S	R	I	R	S	S	S	S	-	-	-	-
SAG4	S	R	S	R	S	S	S	S	S	S	-	-	-	-
SAG5	S	S	S	R	S	S	S	S	S	S	-	-	-	-
SAG6	S	S	S	S	I	S	S	S	S	S	-	-	-	-
SAG7	S	S	S	S	R	S	S	S	S	S	-	-	-	-
SDY1	S	S	S	S	S	S	S	S	S	S	-	-	-	-
SDY2	S	S	S	S	R	S	S	S	S	S	-	-	-	-
SDY3	S	S	S	S	S	S	S	S	S	S	-	-	-	-
SDY4	S	S	S	R	S	S	S	S	S	S	-	-	-	-
SDY5	S	S	S	R	I	S	S	S	S	S	-	-	-	-
SDY6	S	S	S	R	S	S	S	S	S	S	-	-	-	-
SDY7	R	R	R	S	R	S	S	S	S	S	-	-	-	-
SUB1	S	S	S	R	S	R	S	S	S	S	-	-	-	-
SUB2	R	S	S	R	S	S	S	S	S	S	-	-	-	-
SUB3	R	R	R	R	R	S	R	S	S	R	-	-	-	-
SUB4	R	R	S	R	S	S	S	S	S	R	-	-	-	-
SUB5	S	R	S	S	S	S	S	S	S	S	-	-	-	-
SUB6	R	R	S	S	R	S	S	S	S	S	-	-	-	-
SUB7	S	S	S	S	S	S	S	S	S	S	-	-	-	-
SUB ATCC	R	R	S	R	R	S	R	S	S	S	-	-	-	-

S = Sensitive; I = Intermediate; R = Resistant - Please note that we have adapted the format of reporting antibiogram results to the new international standards (CLSI versions VET01S-ED5:2020 and M100-ED32: 2022), P = Penicillin 10 μg; AMP = Ampicillin 10 μg; AMC = Amoxicillin & Clavulanic acid 30 μg; OX = Oxacillin 5 μg; TE = Tetracycline 30 μg; CFX/K = Ubrolexin (Cephalexin 15 μg & Kanamycin 30 μg); KF = Cephalothin 30 μg; RAX = Rifaximin 40 μg; FOX = Cefoxitin 30 μg; EFT = Ceftiofur 30 μg; N = Neomycin 30 μg; K = Kanamycin 30 μg; CN= Gentamicin 10 μg; PB = Polymyxin B 300 μg; ECO 1-7 = Clinical isolates of *E. coli*; SAG 1-7 = clinical isolates of *Streptococcus agalactiae*; SDY 1-7 = Clinical isolates of *Streptococcus dysgalactiae*; SUB 1-7 = Clinical isolates of *Streptococcus uberis*; ATCC = American Type Culture Collection; No = Number

extract of *Antidesma venosum* had the lowest percentage yield of 1.74 %. In general, ethanol was a more effective extractant than acetone for all the four plants, as the percentage yields obtained using ethanol were higher than those obtained using acetone for each plant.

3.2. Antibiotic susceptibility testing

The antibiogram results underscore the importance of routinely conducting antibiogram testing to guide clinical decision-making, in order to prevent treatment failure as well as the risk of AMR development. According to Table 4, 82.14% (23 out of 28) of the clinical isolates tested were resistant to at least one antimicrobial agent used and 17.86% (5) were susceptible to all the antibiotics used. The tests further confirmed that 52.17% (12) of the antibiotic resistant isolates were MDR strains. These include 100% (7) of the *E. coli* strains, 42.86% (3) of *S. uberis*, and 14.29% (1) each of *S. agalactiae* and *S. dysgalactiae* strains (Fig. 1).

The *E. coli* isolates were all (100%, or 7 strains) resistant to ubrolexin and cephalothin, 85.71% (6) to rifampicin, 57.14% (4) to cefoxitin and amoxicillin-clavulanic, 42.86% (3) to neomycin and polymyxin B, 28.57% (2) ceftiofur, 14.29% to tetracycline and gentamicin, and 0% were resistant to kanamycin.

All the streptococcal isolates were resistant to at least one of the antibiotics, except cefoxitin and rifampicin. For *S. agalactiae*, five of the isolates were resistant to at least one of ampicillin, cloxacillin/oxacillin, tetracycline/oxytetracycline and ubrolexin. Two (SAG3 and SAG4) were resistant to at least two antibiotics while only one (SAG3) strain was multidrug-resistant. Similarly, *S. dysgalactiae* (SDY 1-7) had high sensitivity to most of the antibiotics. However, resistance to tetracycline was observed in SDY5, while the only multidrug resistant isolate was SDY7. The *S. agalactiae* and *S. dysgalactiae* strains were relatively sensitive to all the beta-lactam antibiotics used.

For *S. uberis* (SUB 1-7), the pattern was quite varied. The strains were all sensitive to rifampicin, cefoxitin and ampicillin, apart from SUB3 which was resistant to seven antibiotics, including all the beta-lactam antibiotics except cefoxitin. All the isolates were resistant to at least one antibiotic, except SUB7. Three of the isolates (SUB3, SUB4 and SUB6) were multidrug-resistant just like the *S. uberis* reference strain. The susceptibility pattern of the isolates varied by species but the environmental organisms were generally more resistant to antibiotics than the contagious ones.

3.3. Minimum inhibitory concentration and total antibacterial activity

The term Minimum Inhibitory Concentration (MIC) refers to the lowest concentration of an antibiotic substance which, when subjected to strictly controlled scientific conditions, effectively inhibits any visible growth of a specific strain of microorganism (Phillips et al., 1998). For the purposes of this study, the categorization of antimicrobial efficacy of the plant extracts, as delineated by Kuete (2010) was adopted. This categorization involves a "good" MIC, denoted as MIC $<$ 0.1 mg/mL, "moderate" with 0.1 $\leq$ MIC $\leq$ 0.63 mg/mL, and "weak" with MIC $>$ 0.63 mg/mL.

The acetone extracts of all the plants were more active than all their ethanol counterparts (Table 5). The MICs obtained varied with individual plants and organisms, with some plants showing marked activities and some organisms showing susceptibility despite their AMR nature. When observed against individual strains the acetone extract of *S. lancea* demonstrated good activity against 80% (24) of the 30 organisms tested, followed by the ethanol extract (60%) and

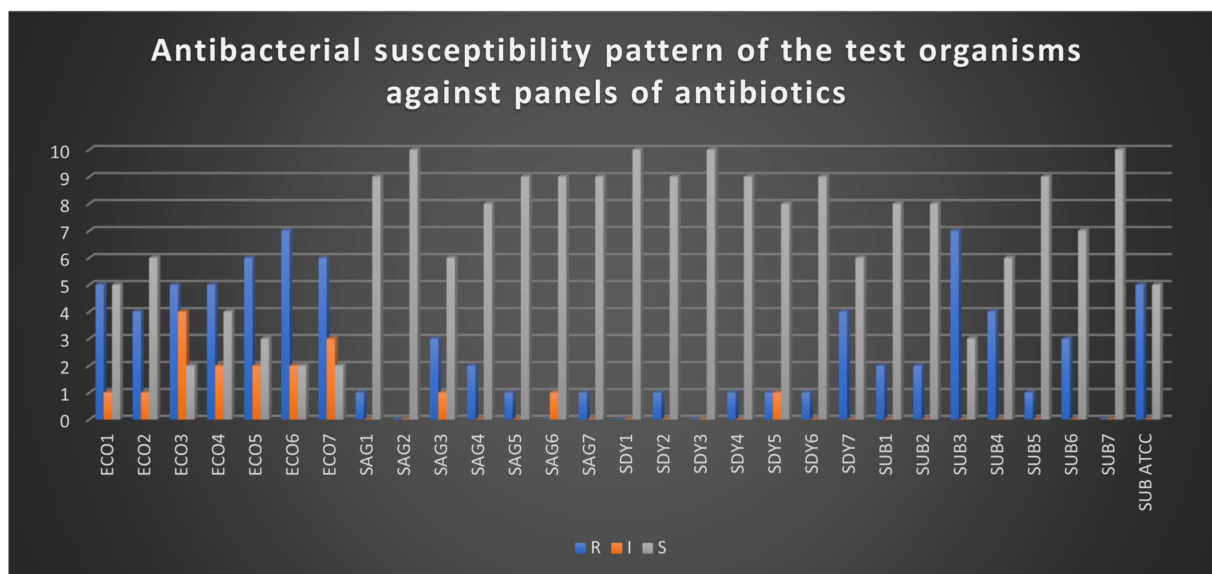

Fig. 1. Antibacterial susceptibility pattern of the test bacteria
S = Sensitive; I = Intermediate; R = Resistant; ECO 1-7 = Clinical isolates of *E. coli*; SAG 1-7 = clinical isolates of *Streptococcus agalactiae*; SDY 1-7 = Clinical isolates of *Streptococcus dysgalactiae*; SUB 1-7 = Clinical isolates of *Streptococcus uberis*; ATCC = American Type Culture Collection.

acetone extract of *A. venosum* (47%). The least active extract was the ethanol extract of *E. caffra* which demonstrated good activity against only one (3.33%) isolate (ECO3). The lowest MIC was 0.01 ± 0.00 mg/mL which was demonstrated by acetone and ethanol extracts of *S. lancea* against SUB1, ECO2, ECO4 and ECO5, while the highest MIC (2.50 ± 0.00 mg/mL) obtained in this study was observed largely against SUB6 by all the plants except *S. lancea*, making SUB6 the least susceptible isolate. All extracts had good to moderate activity against the reference *E. coli* strain (ATCC 25922). Only the ethanol extract of *S. lancea* had moderate activity against *S. uberis* (ATCC 700407), while the rest were only weakly active.

The MIC values of the extracts against the diverse bacterial isolates was in the range of 0.01 - 2.50 mg/mL (Table 6). Only the acetone extract of *S. lancea* had good to moderate activity against all the clinical isolates (MIC = 0.01 – 0.57 mg/mL). The acetone extracts of *S. lancea, A. venosum* and *I. frutescens* had good antibacterial activity (MIC range of 0.01 – 0.08 mg/mL) against the *E. coli* isolates. However, only the acetone extract of *S. lancea* demonstrated good activity

Table 5
MIC range of the extracts in mg/mL based on the group of bacterial isolates.

	MIC range of the extracts (mg/mL)							
Plants	*Searsia lancea*		*Erythrina caffra*		*Antidesma venosum*		*Indigofera frutescens*	
Extractants	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
ECO isolates	0.01 - 0.08	0.02 - 0.16	0.08 - 0.16	0.08 - 0.16	0.02 - 0.04	0.08 - 0.31	0.04 - 0.07	0.04 - 0.16
SAG isolates	0.02 - 0.57	0.03 - 0.29	0.07 - 1.25	0.16 - 1.15	0.07 - 0.57	0.07 - 1.25	0.07 - 1.15	0.08 - 1.25
SDY isolates	0.02 - 0.46	0.03 - 2.35	0.78 - 1.56	0.31 - 2.35	0.02 - 0.78	0.02 - 0.78	0.16 - 0.78	0.02 - 1.56
SUB isolates	0.01 - 0.09	0.01 - 0.16	0.04 - 2.50	0.16 - 2.50	0.04 - 2.50	0.16 - 2.50	0.04 - 2.50	0.09 - 2.50
All *Streptococcus* isolates	0.01 - 0.57	0.01 - 2.35	0.04 - 2.50	0.16 - 2.50	0.02 - 2.50	0.02 - 2.50	0.04 - 2.50	0.02 - 2.50
All isolates	0.01 - 0.57	0.01 -2.35	0.04 - 2.50	0.08 - 2.50	0.02 - 2.50	0.02 - 2.50	0.04 - 2.50	0.02 - 2.50

MIC = minimum inhibitory concentration; SAG = *Streptococcus agalactiae*; SDY = *Streptococcus dysgalactiae*; SUB = *Streptococcus uberis*; Strep = *Streptococcus* species; ECO = *Escherichia coli*; bold = the best range and/or the range that is less than 0.1 mg/mL

Table 6
Average MIC of the extracts in mg/mL based on the group of bacterial isolates ± SD.

	Average MIC of the extracts (mg/mL) ± SD							
Plants	*Searsia lancea*		*Erythrina caffra*		*Antidesma venosum*		*Indigofera frutescens*	
Extractants	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
ECO isolates	0.02 ± 0.03***	0.09 ± 0.06	0.10 ± 0.04	0.15 ± 0.03	0.03 ± 0.01***	0.15 ± 0.08	0.05** ± 0.02	0.09 ± 0.05
SAG isolates	0.10 ± 0.21	0.08 ± 0.09	0.34 ± 0.41	0.48 ± 0.33	0.20 ± 0.18	0.44 ± 0.42	0.26 ± 0.40	0.28 ± 0.43
SDY isolates	0.10 ± 0.13	0.08 ± 0.08*	0.39 ± 0.32	0.32 ± 0.24	0.20 ± 0.17	0.40 ± 0.28	0.27 ± 0.26	0.43 ± 0.28
SUB isolates	0.06 ± 0.03	0.08 ± 0.06	0.85 ± 1.14	1.36 ± 1.13	0.85 ± 1.14	0.94 ± 0.81	0.68 ± 0.91	0.90 ± 0.85
All *Streptococcus* isolates	0.12 ± 0.17	0.24 ± 0.52	0.77 ± 0.77	1.00 ± 0.85	0.51 ± 0.70	0.60 ± 0.58	0.49 ± 0.59	0.61 ± 0.64
All Isolates	0.10 ± 0.15	0.20 ± 0.46	0.60 ± 0.73	0.79 ± 0.82	0.39 ± 0.64	0.48 ± 0.54	0.38 ± 0.54	0.48 ± 0.60

MIC = minimum inhibitory concentration; SAG = *Streptococcus agalactiae*; SDY = *Streptococcus dysgalactiae*; SUB = *Streptococcus uberis*; ECO = *Escherichia coli*; SD = standard deviation; bold = MIC < 0.1 mg/mL; Strep = *Streptococcus* species; * = $p < 0.05$; ** = p = 0.008; *** = $p < 0.001$

Table 7
MIC values of the extracts against each of the bacterial isolates and ATCC strains.

	Mean MIC (mg/mL) ± SD								
Plants	*Searsia lancea*		*Erythrina caffra*		*Antidesma venosum*		*Indigofera frutescens*		Ciprofloxacin (μg/mL)
Extractant	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol	
SAG1	0.02 ± 0.01	0.07 ± 0.02	0.29 ± 0.06	0.57 ± 0.13	0.14 ± 0.03	1.25 ± 0.00	0.16 ± 0.00	0.08 ± 0.00	<0.39
SAG2	0.02 ± 0.00	0.08 ± 0.00	0.29 ± 0.06	0.31 ± 0.00	0.14 ± 0.03	0.31 ± 0.00	0.10 ± 0.04	0.08 ± 0.00	<0.39
SAG3	0.02 ± 0.00	0.03 ± 0.01	0.14 ± 0.03	0.16 ± 0.00	0.14 ± 0.03	0.14 ± 0.03	0.16 ± 0.00	0.14 ± 0.03	<0.39
SAG4	0.02 ± 0.00	0.04 ± 0.01	0.29 ± 0.06	0.57 ± 0.13	0.29 ± 0.06	0.57 ± 0.13	0.07 ± 0.02	0.14 ± 0.03	<0.39
SAG5	0.02 ± 0.00	0.04 ± 0.00	0.07 ± 0.02	0.29 ± 0.06	0.08 ± 0.00	0.07 ± 0.02	0.08 ± 0.00	0.16 ± 0.00	<0.39
SAG6	0.02 ± 0.00	0.04 ± 0.01	0.08 ± 0.00	0.29 ± 0.06	0.07 ± 0.02	0.14 ± 0.03	0.08 ± 0.00	0.14 ± 0.03	<0.39
SAG7	0.57 ± 0.13	0.29 ± 0.06	1.25 ± 0.00	1.15 ± 0.26	0.57 ± 0.13	0.63 ± 0.00	1.15 ± 0.26	1.25 ± 0.00	25.00 ± 0.00
SDY1	0.02 ± 0.00	0.03 ± 0.00	0.72 ± 0.16	1.56 ± 0.00	0.36 ± 0.08	0.39 ± 0.00	0.78 ± 0.00	0.39 ± 0.00	<0.39
SDY2	0.02 ± 0.00	0.03 ± 0.00	0.78 ± 0.00	0.72 ± 0.16	0.39 ± 0.00	0.78 ± 0.00	0.36 ± 0.08	0.72 ± 0.16	1.56 ± 0.00
SDY3	0.13 ± 0.05	0.09 ± 0.02	1.56 ± 0.00	1.04 ± 0.40	0.02 ± 0.00	0.31 ± 0.00	0.39 ± 0.00	0.37 ± 0.13	<0.39
SDY4	0.39 ± 0.00	0.91 ± 0.32	1.56 ± 0.00	1.56 ± 0.00	0.39 ± 0.00	0.78 ± 0.00	0.16 ± 0.00	0.78 ± 0.00	1.56 ± 0.00
SDY5	0.46 ± 0.16	2.35 ± 0.86	0.78 ± 0.00	0.57 ± 0.13	0.59 ± 0.21	0.02 ± 0.00	0.59 ± 0.21	0.02 ± 0.00	0.78 ± 0.00
SDY6	0.06 ± 0.02	0.12 ± 0.04	0.78 ± 0.00	2.35 ± 0.86	0.78 ± 0.00	0.12 ± 0.43	0.78 ± 0.00	1.56 ± 0.00	0.39 ± 0.00
SDY7	0.39 ± 0.00	0.39 ± 0.00	1.56 ± 0.00	0.31 ± 0.00	0.78 ± 0.00	0.47 ± 0.17	0.78 ± 0.00	0.63 ± 0.00	1.56 ± 0.00
SUB 1	0.01 ± 0.00	0.01 ± 0.00	0.09 ± 0.03	0.12 ± 0.04	0.04 ± 0.00	0.29 ± 0.06	0.09 ± 0.03	0.12 ± 0.04	<0.39
SUB 2	0.08 ± 0.00	0.08 ± 0.00	0.26 ± 0.08	2.50 ± 0.00	0.31 ± 0.00	1.25 ± 0.00	0.31 ± 0.00	1.25 ± 0.00	<0.39
SUB 3	0.08 ± 0.00	0.16 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	1.25 ± 0.00	1.25 ± 0.00	1.25 ± 0.00	12.5
SUB 4	0.04 ± 0.00	0.05 ± 0.02	0.04 ± 0.01	1.25 ± 0.00	0.04 ± 0.00	0.63 ± 0.00	0.04 ± 0.00	0.63 ± 0.00	<0.39
SUB 5	0.09 ± 0.03	0.08 ± 0.00	0.47 ± 0.17	0.47 ± 0.18	0.47 ± 0.19	0.47 ± 0.20	0.47 ± 0.21	0.47 ± 0.22	<0.39
SUB 6	0.08 ± 0.00	0.16 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	<0.39
SUB 7	0.02 ± 0.00	0.04 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.08 ± 0.00	0.16 ± 0.00	0.08 ± 0.00	0.09 ± 0.03	<0.39
SUB ATCC	2.29 ± 0.51	0.57 ± 0.13	2.29 ± 0.51	1.25 ± 0.00	2.50 ± 0.00	1.15 ± 0.26	1.15 ± 0.26	1.25 ± 0.00	50
ECO1	0.02 ± 0.01	0.16 ± 0.00	0.08 ± 0.00	0.16 ± 0.00	0.04 ± 0.00	0.08 ± 0.00	0.07 ± 0.02	0.04 ± 0.00	<0.39
ECO2	0.01 ± 0.00	0.03 ± 0.01	0.08 ± 0.00	0.16 ± 0.00	0.02 ± 0.00	0.16 ± 0.00	0.04 ± 0.00	0.08 ± 0.00	<0.39
ECO3	0.02 ± 0.00	0.08 ± 0.00	0.16 ± 0.00	0.08 ± 0.00	0.02 ± 0.00	0.08 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	<0.39
ECO4	0.01 ± 0.00	0.06 ± 0.02	0.08 ± 0.00	0.16 ± 0.00	0.04 ± 0.00	0.16 ± 0.00	0.06 ± 0.02	0.12 ± 0.04	<0.39
ECO5	0.01 ± 0.00	0.02 ± 0.00	0.08 ± 0.00	0.16 ± 0.00	0.04 ± 0.00	0.16 ± 0.00	0.08 ± 0.00	0.12 ± 0.04	<0.39
ECO6	0.08 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.04 ± 0.00	0.31 ± 0.00	0.04 ± 0.00	0.16 ± 0.00	<0.39
ECO7	0.02 ± 0.00	0.12 ± 0.04	0.08 ± 0.00	0.16 ± 0.00	0.02 ± 0.00	0.08 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	<0.39
ECO ATCC	0.08 ± 0.00	0.10 ± 0.04	0.18 ± 0.04	0.18 ± 0.04	0.08 ± 0.00	0.40 ± 0.00	0.10 ± 0.04	0.18 ± 0.04	<0.39

MIC = minimum inhibitory concentration; SAG 1-7 = *Streptococcus agalactiae*; SDY 1-7 = *Streptococcus dysgalactiae;* SUB 1-7 = *Streptococcus uberis*; ECO 1-7 = *Escherichia coli*; ATCC = American Type Culture Collection; SD = standard deviation; bold = MIC < 0.1 mg/mL

against *S. uberis* isolates and good to moderate activity against the *S. dysgalactiae* isolates. Both extracts of *S. lancea* and the acetone extract of *A. venosum* had good to moderate activity (MIC range of 0.02 – 0.57 mg/mL) against *S. agalactiae* isolates, just like all the plant extracts showed against the *E. coli* isolates. The overall MIC range for all the extracts against the *E. coli* isolates was 0.01 – 0.31 mg/mL. These values showed that the extracts exhibited good to moderate activities against these multidrug resistant (MDR) *E. coli* isolates.

The data in Table 6 were used to conduct a one-way analysis of variance (ANOVA) using IBM SPSS Statistic 27, comparing the mean MIC of each solvent extract of each plant against each species of the isolates. For example, the mean MIC of the acetone extract of *Searsia lancea* against *S. agalactiae*, was compared with the mean MIC of acetone extracts of the remaining three plants. This was repeated with all the acetone extracts and ethanol extracts. For *S. agalactiae*, there was no significant difference between the mean MICs of both acetone and ethanol extracts of all the plants when compared against one another ($p = 0.247 - 1$). The same was obtained with acetone extracts against *S. dysgalactiae* ($p = 0.254 - 1$) and ethanol extracts against *E. coli* ($p = 0.344 - 1$). Contrarily, for *S. dysgalactiae*, the difference between the mean MICs of the ethanol extract of *S. lancea* and *I. frutescens* was significant ($p = 0.026$). In the same vein, against *E. coli*, the differences between the mean MIC of the acetone extract of *E. caffra* and the other three plants were also significant with $p < 0.001$ (*S. lancea* and *A. venosum*) and $p = 0.008$ (*I. frutescens*), but all other comparisons were not significant with $p > 0.05$.

As depicted in Table 7, the determination of the average MIC was undertaken by computing the mean of the MIC values of each extract against each isolate of the respective species. Though the lowest average MIC value (0.02 mg/mL) was obtained with the acetone extract of *S. lancea* against *E. coli*, only its ethanol extract had average MICs less than 0.1 mg/mL against three out of the four species of the isolates. The acetone extract of *S. lancea* exhibited average MICs below 0.10 mg/mL against *E. coli* and *S. uberis* strains, whereas the ethanol extract displayed this activity against *S. dysgalactiae* in addition to *E. coli* and *S. uberis* isolates. This suggests its wider range of good activity against the species than its more active acetone counterpart. The highest average MIC (1.36 mg/mL) was obtained with the ethanol extract of *E. caffra*. Except for both extracts of *E. caffra* and the ethanol extract of *A. venosum*, all the extracts had average MICs below 0.1 mg/mL against the *E. coli* strains. The lowest average MIC (0.21 mg/mL) against the *S. dysgalactiae* group was obtained with the acetone extract of *S. lancea*, which makes *S. dysgalactiae* appear to be the least susceptible to all the extracts on average. The MIC values of ciprofloxacin against the tested strains ranged between <0.39 and 50 μg/mL.

Total antibacterial activity (TAA) is a quantitative measure of the potency of plant extracts, and considers both the MIC (mg/mL) and the extract yield (mg/g) (Eloff, 2004). In this study, these values had distinctive patterns (Table 8). The acetone extract of *S. lancea* demonstrated the highest TAA value of 2 396.47 mL/g against *E. coli*, while the ethanol extracts registered TAA values of 1 905.69 mL/g (*S. uberis*), 1 873.39 mL/g (*S. agalactiae*), and 1 754.44 mL/g (*E. coli*). The ethanol extract of *S. lancea* demonstrated higher efficacy against three of the four species of isolates. Also, when considering individual isolates, the ethanol extract of *S. lancea* exhibited an exceptional TAA value of 15 790 mL/g against ECO4 (Table 9). It had TAA values above 1 000 mL/g against 83.33% (25 out of 30) of the organisms which is more than 56.67% (17) by its acetone extracts. This suggests the ethanol extract is more efficacious than the acetone extract. Generally, the extracts appeared to be least efficacious to the reference strain of *S. uberis*.

Table 8
Total antibacterial activity (TAA) of the extracts (mL/g) based on the average MIC of the groups of the test bacteria.

Total antibacterial activity (TAA) of the extracts (mL/g) based on the average MIC of the groups of bacteria								
Plants	*Searsia lancea*		*Erythrina caffra*		*Antidesma venosum*		*Indigofera frutescens*	
Extractants	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
ECO isolates	2 396.47	1 754.44	341.25	321.06	553.64	205.92	561.89	981.17
SAG isolates	590.43	1 873.39	101.95	99.97	85.17	68.20	115.50	295.83
SDY isolates	277.14	281.96	31.74	41.17	36.80	73.90	54.14	131.70
SUB isolates	1 018.50	1 905.69	41.37	35.15	20.51	32.38	43.86	93.30
All Strep isolates	477.42	651.45	45.81	47.81	34.21	50.78	60.09	138.30
All Isolates	596.92	772.94	58.47	60.74	44.70	62.57	77.36	176.13

TAA = total antibacterial activity; MIC = minimum inhibitory concentration; SAG = *Streptococcus agalactiae*; SDY = *Streptococcus dysgalactiae*; SUB = *Streptococcus uberis*; ECO = *Escherichia coli*; mL/g = millilitre per gram; bold = TAA > 1000 mL/g; Strep = *Streptococcus* species

3.4. Cytotoxicity and selectivity index

Table 10 shows the results of assays carried out to evaluate the effects of the selected plant extracts on two different cell types: bovine dermis (BD) cells and Vero cells (derived from the kidney of an African green monkey). The evaluated parameters include the LC_{50} (lethal concentration that kills 50% of cells) and the mean selectivity index (SI) for each plant extract.

According to Kuete (2010), a plant extract is considered to be cytotoxic when the LC_{50} is 0.02 mg/mL and below. The highest LC_{50} value obtained in this study against BD cells was > 1 mg/mL by the ethanol extract of *S. lancea* and acetone extract of *E. caffra* and the lowest value was 0.1 mg/mL. The highest values obtained from the plant extracts against Vero cells was 0.51 mg/mL by the acetone extract of *E. caffra*, while the lowest was < 0.075 mg/mL by both extracts of *A. venosum* and the acetone extract of *S. lancea*. This shows that all the plant extracts were relatively non-cytotoxic to both BD and Vero cells, as all the LC_{50} of all the extracts against both cells were above the suggested 0.02 mg/mL toxic concentration. In general, the extracts were less toxic to the BD cells than the Vero cells.

A selectivity index (SI) value greater than 1 implies that the extract is more toxic to the pathogen than to the mammalian cells used for cytotoxicity testing. It is a numerical value that provides a measure of the selectivity or specificity of a compound or treatment's effect on different biological targets or organisms. The higher the SI value, the more promising is the activity of the plant extract as it is not likely to be owing to general toxicity. Therefore, the higher the SI, the higher the potential of the plant extract to be developed as a safe herbal product.

Table 9
Total antibacterial activity (TAA) of the extracts against the bacterial strains in mL/g.

Plants	*Searsia lancea*		*Erythrina caffra*		*Antidesma venosum*		*Indigofera frutescens*	
Extractant	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
% Yield (g/g)	5.82	15.79	3.51	4.77	1.74	3.03	2.97	8.41
SAG1	2 512.23	2 208.39	122.37	83.25	121.68	24.24	190.38	1 078.21
SAG2	3 174.55	2 024.36	122.37	152.4	121.68	96.81	285.58	268.69
SAG3	2 910.00	5 352.54	245.45	305.77	121.68	211.89	190.38	588.11
SAG4	2 910.00	4 406.51	122.37	83.25	60.66	52.88	415.38	588.11
SAG5	2 910.00	4 048.72	490.91	166.3	223.08	423.78	380.77	539.1
SAG6	2 910.00	4 406.51	450	166.3	243.36	211.89	380.77	588.11
SAG7	101.57	550.49	28.08	41.63	30.37	48.48	25.92	67.28
SDY1	2 910.00	5 263.33	49.09	30.58	48.56	77.69	38.08	215.64
SDY2	2 910.00	5 263.33	45	66.71	44.62	38.85	82.88	117.62
SDY3	436.5	1 722.55	22.5	45.87	87	96.81	76.15	230.41
SDY4	149.23	173.52	22.5	30.58	44.62	38.85	190.38	107.82
SDY5	127.91	67.33	45	83.25	29.74	1 515.00	50.77	4 205.00
SDY6	997.71	1 353.43	45	20.34	22.31	258.97	38.08	53.91
SDY7	149.23	404.87	22.5	152.4	22.31	64.61	38.08	134.56
SUB 1	5 820.00	15 790.00	385.71	407.69	446.15	105.64	326.37	718.8
SUB 2	746.15	2 024.36	134.65	19.08	55.59	24.24	94.89	67.28
SUB 3	746.15	1,012.18	14.04	19.08	6.96	24.24	23.76	67.28
SUB 4	1 492.31	3 470.33	979.53	38.16	446.15	48.48	761.54	134.56
SUB 5	813.99	2 024.36	74.84	101.71	37.1	64.61	63.33	179.32
SUB 6	746.15	1 012.18	14.04	19.08	6.96	12.12	11.88	33.64
SUB 7	2 910.00	4 048.72	225	305.77	223.08	194.23	380.77	924.18
SUB ATCC 700407	25.4	275.57	15.32	38.16	6.96	26.44	25.92	67.28
ECO1	2 512.23	1 012.18	450	305.77	446.15	388.46	415.38	2 156.41
ECO2	5 820.00	4 833.67	450	305.77	870	194.23	761.54	1 078.21
ECO3	2 910.00	2 024.36	225	611.54	870	388.46	761.54	2 156.41
ECO4	11 640.00	2 699.15	450	305.77	446.15	194.23	507.69	718.8
ECO5	5 820.00	7 895.00	450	305.77	446.15	194.23	380.77	718.8
ECO6	746.15	1 012.18	225	305.77	446.15	96.81	761.54	539.1
ECO7	2 910.00	1 349.57	450	305.77	870	388.46	761.54	2 156.41
ECO ATCC 25922	727.50	1 579.00	195.00	265.00	217.50	75.75	297.00	467.22

ATCC = American type culture collection; % = percentage; g = gram; mL = millilitre; ECO 1-7 = Clinical isolates of *Escherichia coli*; SAG 1-7 = Clinical isolates of *Streptococcus agalactiae*; SDY 1-7 = Clinical isolates of *Streptococcus dysgalactiae*; SUB 1-7 = Clinical isolates of *Streptococcus uberis*; bold = TAA > 1000 mL/g;

Table 10
LC_{50} and mean selectivity index (SI) of selected plant extracts against Vero cells based on average MICs of each group of bacterial isolates ± SEM.

Mean Selectivity index (SI) of selected plant extracts against Vero cells and BD cells ± SD								
	Searsia lancea		*Erythrina caffra*		*Antidesma venosum*		*Indigofera frutescens*	
	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
Vero cells								
LC_{50} (mg/mL)	< 0.075	0.15 ± 0.04	0.51 ± 0.09	0.09 ± 0.01	< 0.075	< 0.075	0.33 ± 0.03	0.27 ± 0.04
Escherichia coli	5.96 ± 4.63	2.83 ± 2.42	5.60 ± 1.60	0.66 ± 0.22	2.71 ± 0.98	0.65 ± 0.30	6.91 ± 1.99	4.37 ± 2.44
Streptococcus agalactiae	3.21 ± 1.38	3.12 ± 1.63	3.28 ± 2.60	0.27 ± 0.16	0.57 ± 0.34	0.38 ± 0.35	2.97 ± 1.56	1.71 ± 1.01
Streptococcus dysgalactiae	1.41 ± 1.64	1.93 ± 2.17	0.52 ± 0.18	0.12 ± 0.09	0.18 ± 0.10	0.17 ± 0.11	0.54 ± 0.25	0.59 ± 0.39
Streptococcus uberis	2.44 ± 2.45	3.99 ± 4.98	3.79 ± 4.98	0.25 ± 0.30	0.75 ± 0.86	0.17 ± 0.16	2.64 ± 3.04	0.98 ± 1.16
Bovine Dermis cells								
LC_{50} (mg/mL)	0.10 ± 0.01	> 1	> 1	0.63 ± 0.04	0.65 ± 0.00	0.48 ± 0.02	0.61 ± 0.01	0.46 ± 0.02
Escherichia coli	7.94 ± 6.17	18.84 ± 16.14	10.99 ± 3.13	4.62 ± 1.53	23.45 ± 8.46	4.18 ± 1.93	12.76 ± 3.67	7.44 ± 4.17
Streptococcus agalactiae	4.28 ± 1.84	20.81 ± 10.85	6.44 ± 5.10	1.89 ± 1.15	4.92 ± 2.91	2.42 ± 2.25	5.48 ± 2.89	2.91 ± 1.72
Streptococcus dysgalactiae	1.89 ± 2.19	12.89 ± 14.49	1.02 ± 0.36	0.81 ± 0.61	1.60 ± 0.84	1.12 ± 0.69	1.01 ± 0.47	1.00 ± 0.66
Streptococcus uberis	3.25 ± 3.28	25.70 ± 33.68	7.42 ± 9.79	2.53 ± 2.89	7.56 ± 7.12	1.84 ± 2.25	4.96 ± 5.57	1.76 ± 1.96

BD = bovine dermis; LC_{50} = lethal concentration; SI = selectivity; MIC = minimum inhibitory concentration; mg/mL = milligram per millilitre; SD = standard deviation

The SI values in Table 10 indicate that the extracts had SI values between 0.81 and 25.70 against the BD cells. The highest SI (25.70) against BD cells was demonstrated by the ethanol extract of *S. lancea* when calculated with its average MIC against *S. uberis* isolates. This was the only extract that demonstrated SI values above 10 against all organisms with SI ranging from 12.89 to 25.70. Only the ethanol extract of *E. caffra* had SI below 1 when used against *S. dysgalactiae* in light of its LC_{50} value. This confirms that nearly all the extracts were more toxic to the tested bacteria than the BD cells.

With regard to the Vero cells, the SI values of the extracts against all the isolates ranged from 0.12 – 5.96. The highest SI values were calculated with the acetone extract of *S. lancea* against *E. coli* while the lowest was observed with the ethanol extract of *E. caffra* against *S. dysgalactiae*. The extracts from *S. lancea* did not exhibit a SI value below 1 for any of the isolates, while the ethanol extract of *E. caffra* and both extracts of *A. venosum* were more toxic to Vero cells than nearly all the extracts, as almost all the SI values were below 1.

Table 11
Selectivity index (SI) of the plant extracts against bovine dermis and Vero monkey cells based on minimum inhibitory concentrations (MICs) against the test bacteria.

Selectivity index (SI) of selected plant extracts against BD and Vero cells																
	Searsia lancea				*Erythrina caffra*				*Antidesma venosum*				*Indigofera frutescens*			
	Acetone		Ethanol		Acetone		Ethanol		Acetone		Ethanol		Acetone		Ethanol	
Bacteria Isolates	BD	Vero	BD	Vero	BD	Vero	BD	Vero	BD	Vero	BD	Vero	BD	Vero	BD	Vero
SAG1	4.32	3.24	13.99	2.10	3.49	1.78	1.10	0.16	4.55	0.52	0.38	0.06	3.91	2.12	5.90	3.46
SAG2	5.45	4.09	12.82	1.92	3.49	1.78	2.01	0.28	4.55	0.52	1.53	0.24	5.87	3.17	1.47	0.86
SAG3	5.00	3.75	33.90	5.08	7.00	3.57	4.04	0.58	4.55	0.52	3.36	0.52	3.91	2.12	3.22	1.89
SAG4	5.00	3.75	27.91	4.19	3.49	1.78	1.10	0.16	2.27	0.26	0.88	0.13	8.53	4.62	3.22	1.89
SAG5	5.00	3.75	25.64	3.85	13.99	7.13	2.20	0.31	8.33	0.96	6.71	1.05	7.82	4.23	2.95	1.73
SAG6	5.00	3.75	27.91	4.19	12.82	6.54	2.20	0.31	9.09	1.05	3.36	0.52	7.82	4.23	3.22	1.89
SAG7	0.17	0.13	3.49	0.52	0.80	0.41	0.55	0.08	1.13	0.13	0.77	0.12	0.53	0.29	0.37	0.22
SDY1	5.00	3.75	33.33	5.00	1.40	0.71	0.40	0.06	1.81	0.21	1.23	0.19	0.78	0.42	1.18	0.69
SDY2	5.00	3.75	33.33	5.00	1.28	0.65	0.88	0.13	1.67	0.19	0.62	0.10	1.70	0.92	0.64	0.38
SDY3	0.75	0.56	10.91	1.64	0.64	0.33	0.61	0.09	3.25	0.38	1.53	0.24	1.56	0.85	1.26	0.74
SDY4	0.26	0.19	1.10	0.16	0.64	0.33	0.40	0.06	1.67	0.19	0.62	0.10	0.39	0.21	0.59	0.35
SDY5	0.22	0.16	0.43	0.06	1.28	0.65	1.10	0.16	1.11	0.13	2.40	0.38	1.04	0.56	2.30	1.35
SDY6	1.71	1.29	8.57	1.29	1.28	0.65	0.27	0.04	0.83	0.10	0.41	0.06	0.78	0.42	0.29	0.17
SDY7	0.26	0.19	2.56	0.38	0.64	0.33	2.01	0.29	0.83	0.10	1.02	0.16	0.78	0.42	0.74	0.43
SUB1	10.00	7.50	100.00	15.00	10.99	5.60	5.38	0.77	16.67	1.92	1.67	0.26	6.70	3.63	3.93	2.31
SUB2	1.28	0.96	12.82	1.92	3.84	1.96	0.25	0.04	2.08	0.24	0.38	0.06	1.95	1.05	0.37	0.22
SUB3	1.28	0.96	6.41	0.96	0.40	0.20	0.25	0.04	0.26	0.03	0.38	0.06	0.49	0.26	0.37	0.22
SUB4	2.56	1.92	21.98	3.27	27.91	14.23	0.50	0.07	16.67	1.92	0.77	0.12	15.64	8.46	0.74	0.43
SUB5	1.33	1.05	6.67	1.92	1.96	1.09	7.00	0.19	8.67	0.16	6.40	0.16	1.85	0.70	1.70	0.58
SUB6	1.28	0.96	6.41	0.96	0.40	0.20	0.25	0.04	0.26	0.03	0.19	0.03	0.24	0.13	0.18	0.11
SUB7	5.00	3.75	25.64	3.85	6.41	3.27	4.04	0.58	8.33	0.96	3.08	0.48	7.82	4.23	5.05	2.97
SUB ATCC	0.04	0.03	1.75	0.26	0.44	0.22	0.50	0.07	0.26	0.03	0.42	0.07	0.53	0.29	0.37	0.22
ECO1	4.32	3.24	6.41	0.96	12.82	6.54	4.04	0.58	16.67	1.92	6.15	0.96	8.53	4.62	11.79	6.92
ECO2	10.00	7.50	30.61	4.59	12.82	6.54	4.04	0.58	32.50	3.75	3.08	0.48	15.64	8.46	5.90	3.46
ECO3	5.00	3.75	12.82	1.92	6.41	3.27	8.08	1.15	32.50	3.75	6.15	0.96	15.64	8.46	11.79	6.92
ECO4	20.00	15.00	17.09	2.56	12.82	6.54	4.04	0.58	16.67	1.92	3.08	0.48	10.43	5.64	3.93	2.31
ECO5	10.00	7.50	50.00	7.50	12.82	6.54	4.04	0.58	16.67	1.92	3.08	0.48	7.82	4.23	3.93	2.31
ECO6	1.28	0.96	6.41	0.96	6.41	3.27	4.04	0.58	16.67	1.92	1.53	0.24	15.64	8.46	2.95	1.73
ECO7	5.00	3.75	8.55	1.28	12.82	6.54	4.04	0.58	32.50	3.75	6.15	0.96	15.64	8.46	11.79	6.92
ECO ATCC	1.25	0.94	10.00	1.50	5.56	2.83	3.50	0.50	8.13	0.94	1.20	0.19	6.10	3.30	2.56	1.50

ECO 1-7 = clinical isolates of *Escherichia coli*; SAG 1-7 = clinical isolates of *Streptococcus agalactiae*; SDY 1 – 7 = clinical isolates of *Streptococcus dysgalactiae*; SUB 1-7 = *Streptococcus uberis*; ATCC = American Type Culture Collection; BD = bovine dermis; LC_{50} = lethal concentration 50%; SI = selectivity

According to Table 11 where SI values of each extract against individual isolates are presented, the ethanol extracts of *S. lancea* had the highest SI values of 100 and 15 against BD and Vero cells, respectively. These values are highly promising and provide strong motivation for further studies on this plant species.

4. Discussion

4.1. Yield

Acetone and ethanol are commonly used solvents in plant extractions due to their polarity and ability to dissolve a wide range of phytochemicals. While acetone is generally the favored choice for extraction because of the observed antibacterial activities of its extracts, ethanol was also chosen as an appropriate solvent for future potential commercial viability as ethanol is preferred to more flammable solvents such as acetone in an industrial setting (Panda et al., 2011). The ethanol extract of *S. lancea* resulted in the highest percentage yield (15.79%), followed by the ethanol extract of *I. frutescens* (8.41%), and the acetone extract of *A. venosum* had the lowest yield (1.74%). This trend of yield variations based on different solvents and plant species is common in extraction studies. Ethanol was a more effective extractant than acetone for all four plants, as ethanol yielded higher percentages of dry extracts. The permeability of cell membranes to ethanol is widely recognized, enabling the extraction of larger quantities of intracellular components in comparison to solvents with lower polarity (Panda et al., 2011). This finding aligns with the general understanding that the choice of solvent can significantly impact the type and quantity of compounds extracted from plant materials.

4.2. Antibiotic susceptibility testing

Bacteria acquire resistance through four primary mechanisms: altering antibiotics, modifying antibiotic target sites, adapting metabolic pathways, and enhancing antibiotic expulsion or blocking entry. These insights strongly underline the correlation between antibiotic usage and the rise of AMR in animal husbandry (McManus, 1997).

In this study, *E. coli* strains displayed multidrug resistance (MDR), surpassing the 46% MDR of *E. coli* isolates reported by My et al. (2023). Similarly, reports from different countries, including Iran, Bangladesh, China, Jordan, and Canada, documented varying but concerning levels of MDR among *E. coli* strains causing clinical mastitis (Awosile et al., 2018; Obaidat et al., 2018; Ahmadi et al., 2020; Lan et al., 2020; Bag et al., 2021). Resistance to tetracycline and gentamicin was observed in 14.29% of *E. coli* isolates. The 14.29% resistance by the *E. coli* strains to gentamicin was lower than the 18.5% resistance reported in China (Lan et al., 2020). In contrast, My et al. (2023) claimed there was no resistance to gentamicin among *E. coli* strains tested. Furthermore, the resistance to tetracycline among the *E. coli* isolates in this study is similar to the 14.6% figure reported in Switzerland (Nüesch−Inderbinen et al., 2019), but quite a bit lower than 57.4% in Iran (Momtaz et al., 2012), 45.4% in Jordan (Obaidat et al., 2018), 34.8% in China (Cheng et al., 2019) and 32% reported by My et al. (2023) in Vietnam. The *E. coli* strains exhibited high resistance rates to beta-lactam antibiotics (75% resistance to four out of five types), with ceftiofur resistance reaching 28.57%. This level of resistance surpasses rates reported in other countries like Vietnam, Canada, France and Switzerland (Awosile et al., 2018; Boireau et al., 2018; Nüesch−Inderbinen et al., 2019; My et al., 2023).

The rise in antibiotic resistance among mastitis-causing bacteria presents significant challenges for both veterinary care and human health. Consequently, careful antibiotic management on farms and prudent use is imperative to control and curb the risk of MDR propelled by excessive antibiotic usage (My et al., 2023). These variations emphasize the global diversity in AMR patterns among *E. coli* strains.

This study further reported MDR in other bacterial strains, which include 42.86% of *S. uberis* isolates, and 14.29% each of *S. agalactiae* and *S. dysgalactiae*. Comparable findings revealed higher MDR rates among *S. uberis* compared to *S. dysgalactiae* and *S. agalactiae*. Notably, resistance patterns varied across studies, with tetracycline and cloxacillin resistance being most common in *Streptococcus* species (Minst et al., 2012).

Resistance patterns also varied for *S. agalactiae*, with notable resistance to cloxacillin/ oxacillin (57.14%), ampicillin (28.57%) and tetracycline (14.29%). The study highlighted cross-resistance implications and discrepancies, particularly within ampicillin-resistant strains, while also comparing resistance rates with reference to prior research (Gao et al., 2012; Mesquita et al., 2019). Some *S. dysgalactiae* isolates were resistant to various antibiotics, with 42.86% resistant to cloxacillin/ oxacillin, 28.57% to tetracycline, and 14.29% each to ampicillin, amoxicillin, and penicillin. Similar findings were reported elsewhere, reinforcing the phenomenon of cross-resistance in bacterial isolates (Kovačević et al., 2021). *Streptococcus uberis* exhibited diverse resistance patterns, with significant resistance to penicillin noted in the SUB3 sample. This is concerning since penicillin and related ß-lactam antibiotics are traditionally effective against streptococcal infections (Monistero et al., 2021).

In order to clarify the antibiotic resistance patterns exhibited by these organisms, biomolecular assays are recommended to identify these genes, particularly in *E. coli* strains. It has been discovered that several strains of each pathogenic bacterial species carry pathogenic genes responsible for encoding their specific resistance to antibiotics (Haenni et al., 2018).

These findings highlight the importance of regular antibiotic susceptibility testing to inform clinical decisions. Future research should focus on understanding the mechanisms underpinning resistance and developing novel therapeutic strategies. The excessive use of antibiotics in dairy farming for non-therapeutic purposes necessitates re-evaluation and rational guidelines, including dosage and withdrawal period adjustments (Sharma et al., 2018).

4.3. Antibacterial activity and total antibacterial activity

The interest in plant extracts with antibacterial properties has surged in recent years, leading to numerous reports on their effectiveness. Plants and their bioactive components are increasingly recognized for their ability to exhibit antibacterial effects. This recognition has led to their potential utilization in treating mastitis (Šukele et al., 2022).

The results collectively underscore the better antibacterial activity of acetone extracts over ethanol extracts in inhibiting the growth of MDR isolates of bovine mastitis origin. Nonetheless, the outcomes also accentuate the presence of considerable variability in antimicrobial activities across strains and plant species. Notwithstanding these variations, it was observed that all plant extracts were active against bacterial isolates, with the acetone extract of *S. lancea* showing better activity compared to its ethanol counterpart. This indicates that the choice of solvent can impact the bioactive compounds extracted from the plants (Eloff, 2000).

A prominent trend emerged wherein acetone extracts consistently had better antibacterial activity than their ethanol counterparts, exerting growth inhibition at concentrations below 0.1 mg/mL against *E. coli* isolates, with the exception of *E. caffra.* All plant extracts demonstrated good to moderate antimicrobial activity against the drug resistant *E. coli* isolates and the ATCC reference strain, with MICs spanning the range of 0.01 to 0.31 mg/mL. This observation suggests that the mechanism of action of these plant extracts and their components bypasses the biochemical and physiological AMR mechanisms of the *E. coli* isolates. The active phytochemical compounds are likely to be unrelated to the classes of the antibiotics used in the antibiogram assay.

Literature search did not reveal any previous reports on the antibacterial activity of these plants against any of the bacterial species of bovine mastitis origin. There are also no reports of these plants against streptococcal isolates or strains of the species tested in this study. It therefore appears this is the first study to report on the antibacterial activity of these plants against these bacteria isolated from cases of bovine mastitis. However, there are a few studies showcasing varying antibacterial activities of these plants against *E. coli* of different origin.

Such reports include the work of Adeyemo et al. (2022) which documented similar antibacterial activity of the acetone extract of *S. lancea* against *E. coli* (ATCC 25922), *E. coli* (ATCC 35218) and a diarrhoeagenic *E. coli* isolate. They also confirmed that the ethanol extract demonstrated good activity against an *E. coli* (ATCC 25922) reference strain. Some studies also reported moderate to weak activities of different extracts of *S. lancea* against other strains of *E. coli* (Mulaudzi et al., 2012; Vambe et al., 2021). Contrarily, Obi et al. (2003) reported that ethanol and water extracts of *S. lancea* demonstrated no antibacterial activity against all the tested bacterial strains at concentrations as high as 40.00 and 50.00 mg/mL. McGaw et al. (2007) documented no antibacterial activity by hexane, methanol and water extracts of *S. lancea* at concentrations below 12.50 mg/mL against *E. coli* (ATCC 35219). These extremely high MIC values reported previously are of little value when compared with recent reports and standards of interpretation of MIC results (Eloff, 2021; Kuete, 2010). The variation in the reported antibacterial activity of *S. lancea* against the same and different pathotypes of *E. coli* is not uncommon, but it underscores the need to standardize various phyto-analytical techniques employed in ethnopharmacological research. The biomolecular characterization and constitution of each strain of the bacteria, which determine their susceptibility patterns, is also a factor that must be considered. It has also been argued that the variation might be because of seasonal shifts and the geolocation of the plant which determines the quantity and quality of the phytochemical constituents of the plant. Plants adjust the activity of their biochemical pathways in response to the specific combination of herbivores, pollinators and microorganisms present in their environment (Baldwin, 2002). Consequently, the chemical composition of an individual plant can change over time as it responds to shifting environmental conditions (Pandey et al., 2011). However, these studies underscore the potential of *S. lancea* in management of MDR *E. coli* in prevention and treatment of bovine mastitis.

Other studies which documented antibacterial activities of some of these plants against *E. coli* strains include that of Adamu et al. (2014) which showed that *I. frutescens* exhibited moderate activity against *E. coli* (ATCC 25922), and Dzoyem et al. (2014) which reported that acetone extracts of *I. frutescens* and *E. caffra* had moderate activity against an *E. coli* strain.

In comparison with the positive control, ciprofloxacin, all plant extracts exhibited lower antibacterial activities, which is to be expected as the activity of a pure compound is likely to be higher than that of a complex plant extract comprising many different compounds. The MIC values of ciprofloxacin against the tested strains ranged between <0.39 and 50 μg/mL.

In terms of the number of good antibacterial activities per strain tested, *S. lancea* was the most active plant against the bacteria, followed by *I. frutescens* and *A. venosum.* The pattern of susceptibility of the bacterial isolates to the plant extracts also appears to be the same, which suggests that the biochemical and physiological composition of each species determines their vulnerability to the extracts. The *E. coli* isolates were the most susceptible, followed by *S. uberis* and *S. agalactiae. Streptococcus dysgalactiae* isolates demonstrated the least susceptibility. This pattern is a reversal of their susceptibility pattern to conventional antibiotics as seen in their antibiotic susceptibility testing results in Table 4. This suggests that biochemical and physiological processes responsible for their AMR characteristics appear not to interfere with mode and mechanism of actions of the plant extracts. This underscores the need for further research to discover factors responsible for this phenomenon. It is also an interesting observation considering the fact that this study presents potential alternative management therapy to this MDR bacterial species.

The inhibitory potential of each extract varied against different *Streptococcus* species, highlighting the importance of considering specific bacterial targets when assessing the effectiveness of plant extracts. The holistic measure of antibacterial activity, known as "Total Antibacterial Activity" (TAA), is predicated on the extraction yield in milligrams per gram of plant material and the MIC, denominated in millilitres per gram (mL/g). Consequently, similar to the previous study by Akinboye et al. (2023), the ethanol extract of *S. lancea* emerges as an especially suitable candidate for compound isolation and bioprospecting research.

4.4. Cytotoxicity and selective index

The kidney is a major organ involved in the excretion of most pharmacological substances administered parenterally. Therefore, cytotoxicity assays involving the use of Vero monkey kidney cells are commonly employed in natural products screening. According to Kuete (2010) a plant extract is considered to be cytotoxic when the LC_{50} is 0.02 mg/mL and below. Based on this, all the plant extracts appear to be non-cytotoxic to both BD and Vero cells, as all the LC_{50} values of all the extracts against both cells were above the 0.02 mg/mL limit.

The acetone extract of *A. venosum* had the lowest LC_{50} value which was similar to the cytotoxic activity obtained in the previous study (Akinboye et al., 2023). Although the LC_{50} values obtained in the previous study were higher than the values recorded in this study, the LC_{50} values (0.075 – 0.33 mg/mL) obtained in this study confirm that the plant extracts are relatively non-cytotoxic to the Vero cells. The variation in plant chemical constituents at different seasons of the year may account for this discrepancy.

In agreement with this study, some reports have documented *S. lancea* extracts to be relatively non-cytotoxic to Vero cells (Tshidzumba, 2016; Adeyemo et al., 2022; Akinboye et al., 2023). at concentrations between 0.05 – 0.79 mg/mL. (Dzoyem et al., 2014) reported LC_{50} values of 0.02 and 0.08 mg/mL for *E. caffra* and *I. frutescens*, respectively. Though these values are lower compared to the findings of this study, they further confirm that the plants are relatively non-cytotoxic to Vero cells.

The management of bovine mastitis often includes the use of pharmacological agents such as disinfectants, cleaning agents and teat dips, which are applied on the skin surfaces of the animals. Plant extracts intended for such use should be evaluated for their cytotoxic activity against bovine dermis (BD) cells to determine their safety when used for external application.

In this study, the lowest LC_{50} value of 0.10 mg/mL obtained against BD cells was 400% more than the cut-off concentration suggested for non-cytotoxic substances. This suggests that the plant extracts are generally non-cytotoxic to BD cells. Furthermore, generally, all the plant extracts in this study were more toxic to Vero cells than the BD cells. The study also revealed that the acetone extract of *S. lancea* demonstrated more toxicity than its ethanol counterpart to the BD cells, but another study concluded that both extracts of *S. lancea* were toxic to BD cells, and that the ethanol extracts were more toxic than the acetone extracts (Tshidzumba, 2016).

A literature search did not reveal any published report on the cytotoxicity of acetone and ethanol extracts of *Antidesma venosum, E. caffra* and *I. frutescens* against BD cells, therefore this study appears to be the first to report data on their cytotoxicity to BD cells. Ethanol extracts of the studied plants generally showed higher LC_{50} values than acetone extracts, implying lower toxicity. Similar findings by

Mwangomo et al. (2012) support the preference for ethanol extracts, which are safer for industrial use due to their reduced flammability and danger in larger quantities. This comparison holds importance for potential commercial applications. However, *in vitro* cellular toxicity might not accurately predict *in vivo* toxicity due to factors like gut interactions and bioavailability. Therefore, animal toxicity testing is needed to ensure the safety of plant extracts (Adamu et al., 2014).

A Selectivity Index (SI) value above 1 indicates that a plant extract is more toxic to the targeted pathogen compared to the mammalian cells employed in cytotoxicity testing. A higher SI value signifies greater promise in the extract's effectiveness, as it suggests that the observed activity is not primarily due to overall toxicity. Therefore, a higher SI value corresponds to increased potential for the plant extract to be developed into a safe herbal product. When the SI value exceeds 1, it indicates the plant extract's preferential harm to the pathogen over mammalian cells, supporting its potential for safe product development (Dzoyem et al., 2016).

The SI range obtained by *S. lancea* against Vero cells and BD cells supports their usage in both oral and external applications in traditional medicine (Kose et al., 2015; Mabogo, 2012; Mulaudzi et al., 2012). It is interesting to know that both extracts of *S. lancea* which showed the most promising antibacterial activity against all the organisms tested, also had very promising mean SI ranges with values between 1.38 and 25.70, against both Vero and BD cells. Though the best antibacterial activity was shown by the acetone extract of *S. lancea*, the ethanol extract had the best safety profile. In addition to this, the lower flammability and reduced handling hazards when working with larger quantities of ethanol compared to acetone, as highlighted by Panda et al. (2011), makes the ethanol extract of *S. lancea* the extract of choice for future studies as commercialization of extract preparations is one of the goals of this research.

5. Conclusions

This study aimed to investigate the antibacterial activities of acetone and ethanol extracts of four specific plants against antibiotic resistant strains of *E. coli, S. uberis, S. agalactiae* and *S. dysgalactiae* implicated in bovine mastitis. It was found that the crude extracts exhibited substantial antibacterial activity against most of the antibiotic resistant *E. coli* and streptococcal strains, often surpassing their susceptibility to standard strains used for comparison. Remarkably, a link between bacterial resistance to antibiotics and susceptibility to these plant extracts was noted. Among the plants studied, *S. lancea* demonstrated the most potent antibacterial activity, indicating its potential as a broad-spectrum antibacterial agent against these mastitis-related strains. Importantly, it did so without causing significant harm to cells at moderate concentrations, with ethanol extracts of *S. lancea* showing better efficacy compared to acetone extracts. This suggests that ethanol should be the preferred choice for large-scale plant material extraction.

Future research will delve into the antibiofilm, quorum quenching, antioxidant, and anti-inflammatory activities of these extracts and their components. This will pave the way for the development of sustainable natural products for bovine mastitis management. Key compounds will be isolated and characterised from the ethanol extract of *S. lancea* and potential synergistic interactions between different plant extracts and their components will be explored.

However, it is important to acknowledge the study's limitations, including the focus on laboratory-based testing. Further research is needed, including studies on live dairy cows, the development of formulations for udder application, and a deeper understanding of the mechanisms of action of these plant extracts. Additionally, research should explore the plants' broader pharmacological and antibacterial activities, isolate their active phytochemicals, describe their biomolecular activities, and adapt them for practical veterinary use. In conclusion, this study underscores the urgency of addressing AMR in animal husbandry and the potential of natural products in this endeavour.

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Compliance with ethical standards

The study was approved by the Research Ethics Committee of the Faculty of Veterinary Science, University of Pretoria (Certificate REC038-22).

Declaration of competing interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Ayodele O. Akinboye: Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Rasheed O. Adeyemo:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Joanne Karzis:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Inge-Marie Petzer:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Lyndy J. McGaw:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing.

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