In-Vitro Antimicrobial Effects of Plants Use Limpopo, South Africa Against Bovine Mastitis

Dr Jacobus Kori Madisha

Limpopo Education,84 Limpopo Street, Modimolle, South Africa

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ABSTRACT

Bovine mastitis rests to remain the greatest expensive infection to the dairy agriculturalists. It controls in Limpopo as unique of the utmost predominant infections in dairy cattle amongst the dairy farms. The current study is an in vitro Antimicrobial activities of ten therapeutic plants alongside bovine udder inaccessible bacterial pathogens. Water, acetone, ethanol and methanol abstracts of eleven plants were examined by microdilution technique. All plants extracts displayed noteworthy activities against Staphylococcus aureus, Staphylococcus epidermidis and E.coli and fungi respectively. With mic ranging between 0.098-3.125 both bacterial and fungi, with biofilm of above 50% of premature biofilm. All plants tested to be non-toxic.

Key words: Bovine mastitis pathogens, microdilution method, antibacterial, antibiofilm, antifungal,

INTRODUCTION

Mastitis remains towards be amongst the priciest infections to the dairy productiveness, and yearly monetary damages recognised to this infection in the United States are projected to method $5 billion. Amongst cattle illnesses, bovine mastitis is a severe delinquent which disturbs the elementary revenue of the farmers reducing their dairy sources. Global, mastitis is connected with monetary damages of $65 billion yearly. It undesirably distresses milk production whereby damages due to subclinical mastitis are further severe than those owed to clinical cases. Controlling subclinical mastitis can decrease the losses in milk making significantly. Reduced milk manufacture and superiority, as well as veterinary expenditures, all contributes to these monetary losses [1]. Clinical and subclinical cases of mastitis are normally preserved with antimicrobials both intramammarily and parenterally [8].The use of antimicrobials complete extensive phases has stimulated the improvement of multidrug resistant strains, which has caused in the use of acquisitive quantities of antimicrobials, producing the hazard of growing quantities of medication residues in milk, a potential biohazard [9].Therapeutic plants consume remained castoff for centuries in evolving countries as substitute usage to health complications. South Africa has a miscellaneous flora and a rich custom in the usage of therapeutic plants for antimicrobial applications.

Drugs from plants are relatively effortlessly accessible, economical, safe and efficient with potentially fewer side effects [10] . Herbals which have stayed particular for therapeutic use above thousands of years comprise the mainly noticeable preference of investigation the contemporary examine for curatively useful novel medications such as anticancer treatments [21] , antimicrobial drugs [16,20] , and antihepatotoxic compounds. Nonetheless, such plants would be scrutinised to improved comprehend their properties, wellbeing and effectiveness [31]. Plants are generally readily accessible, cheap, effective, and are an significant source of possibly beneficial structures [30] .

In South Africa specifically in Limpopo ethnoveterinary observes are exact collective in communities. Furthermost of the methods of the farmers are constructed on empiric acquaintance with noteworthy outcomes in cattle. A little enthnoveterinary survey previous to this study was commenced amongst recognised farmers approximately their attentiveness in plants use and cure of their cattle sources. Best of them articulated a aspiration to learn further around the appropriate use and presentation of ethnoveterinary follows as these were carefully, communally and ethnically more satisfactory for ostracised communities. The current study was

presumed to examine the properties of water, acetone, ethanol and methanol extracts of Senna italica , Euphorbia tirucali, Aloe zebrine, Malva parviflora, Sclerocarya birrea, Aloe marlothii , Peltophorum africanum, Euphorbia petricola, Cannabis Sativa and Elephantorrize elephantine**.**

Description of study area

The study was conducted in five local municipalities Elias Motswaledi, Ephraim Mogale,Tubatse, Fetakgomo and Makhuduthamaga of the Sekhukhune District, Limpopo Province, South Africa. Geographically, Sekhukhune District lies between 24°50′S and 29°50′E (Figs 1 and 2). The district is located in the south east part of Limpopo Province, and covers an area of 13 528 km^2, making it the largest district in the province. A large portion of the district is identified as rural areas. Semenya et al. (2013) [19] noted that the high floristic diversity of the area coupled with high unemployment rates resulted in a heavy reliance on natural resources such as plants to meet livelihood needs.

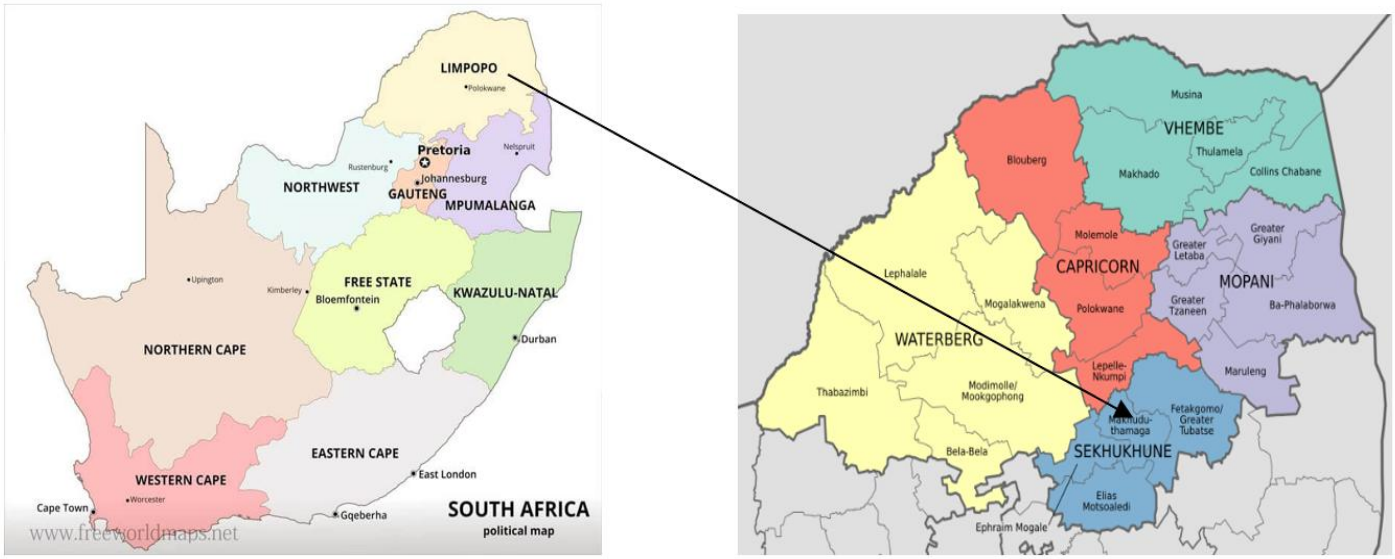

Figure 1. South Africa (www.freeworldmaps.net) and Limpopo province

Data Collection.

The ethnobotanical study was accepted out with the support of some associates of the Sekhukhune Association of Traditional Healers, Limpopo, South Africa a subunit of the South African traditional healers associations. Participants were identified by communal leaders, whose authorisation was sought beforehand scheduled into the study area. A total of 35 traditional healers were interviewed between 1 April 2021 and 30 September 2021. The increase sampling is an method for finding information-rich key informers, who are then communicated and cross-examined, following which they in turn direct the interviewer to other knowledgeable potential respondents (Patton, 1990).Contributors were knowledgeable of the purposes of the study and individual appointments were finished to their families and preparation sites. A previous knowledgeable consensus form (translated into the local Sepedi) was assumed to the accomplices to sign afterward the intentions of the survey had been clarified.

MATERIALS AND METHODS

Plant collection

Fresh plant parts of Senna italica , Euphorbia tirucali, Aloe zebrine, Malva parviflora, Sclerocarya birrea, Aloe marlothii , Peltophorum africanum, Euphorbia petricola, Cannabis Sativa and Elephantorrize elephantine were collected arbitrarily from the gardens and villages of Sekhukhune district, Limpopo, South Africa. The identities of plants were established by Prof Mgda Nel and the voucher specimen of the plant was preserved in HGWJ Schweickerdt Herbarium of the University of Pretoria.

Preparation of Crude Extracts

100 grams of dehydrated plant quantifiable was extracted with 200 ml of water, acetone, ethanol and methanol kept on a rotary shaker for 7 days. Subsequently, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume.

Bacterial strains

Gram-positive bacteria (Staphylococcus aureus, ATCC 29213, Staphylococcus epidermidis ATCC 35984) and gram-negative bacteria (E.coli, ATCC 25922). Two fungous species were used to test the antifungal activity of the plant extracts, namely the yeast species Candida albicans and as well as a mould species Aspergillus fumigatus which were implicated in bovine mastitis

Determination of MIC (micro dilution)

The antimicrobial activities of the herb extracts was studied by means of the micro dilution bioassay as describe [7].MIC of the herbal abstracts was Identify. The lower the MIC is the enhanced is the activities [25]. Antimicrobial activity of herbal abstracts take remained categorised as worthy (MIC< 0.1 mg/mL), reasonable ($0.1 \leq$ MIC ≤ 0.625 mg/mL) and insufficient (MIC >0.625 mg/mL) [26].

Antifungal assay to determine the minimum inhibitory concentration (MIC)

The serial microplate dilution method developed by Eloff (1998b) modified by Masoko and Eloff (2005) was used to determine the MIC values for plant extracts against fungal strains.

Cytotoxicity assay

The cytotoxicity study was conducted by means of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric measure as pronounced [11,12].

Antibiofilm development formation

The technique of [23] remained deployed to examine the plant extracts potential to inhibit development of microbial cell mass and attachment. The biomass was quantified with the changed crystal violet discolouration technique of [14].

Inhibition of pre-formed biofilm

The capability of plant abstracts to inhibit extra formation and or obliteration of cell quantity was also examined. The biofilm biomass was measured by means of the changed crystal violet (CV) discolouration test [14].

Crystal violet staining assay

The technique of [23] was employed for this assay, a modification of [14]. In brief, sterile distilled water was used to wash microtitre plates, air desiccated and also oven dried for 45 minutes in an oven set at 60 ^{0}C. A 100 µl of 1% crystal violet was used to stain the wells of the plate, incubation that takes 15 min and later, the plates were cleaned three times with sterile distilled water to get rid of unreactive stain. At this level, biofilm was observed as purple ring by the side of the wells. The measureable assessment of biofilm development was determined by adding 125 µl of ethanol, this is to remove the stain in the wells. A 100 µl aliquot of the ethanol was withdrawn to a new sterile plate and the absorbance was determine using a microplate reader at 590 nm. The average absorbance was determined for each sample, and their respective proportion inhibition of biofilm calculated using the formula below [23]:

$$\text{Percentage (\%) inhibition} = \frac{\text{ODNegative control} - \text{ODExperimental} \times 100}{\text{ODNegative control}}$$

RESULTS AND DISCUSSION

Table:1 MIC of the pants

Plant Species Native Names	Plant part	Solvent	E.coli	S.ureus	S.epidermidis	A.fumigatus	Candida albicans
Elephantorrize elephantina Mositsane	Roots	MeOH	0.098	0.39	0.78	0.098	0.78
		EA	0.78	3.125	0.195	3.125	0.39
		AC	3.125	6.25	0.78	0.195	0.39
		H2O	0.098	0.195	1.56	0.098	0.78
Cannabis Sativa Motekwane	Leaf	MeOH	0.39	0.195	0.39	3.125	0.39
		EA	1.56	0.098	0.78	0.195	0.195
		AC	3.125	0.098	0.098	0.098	0.195
		H2O	3.125	0.195	0.39	0.78	0.195
Euphorbia petricola	roots	MeOH	0.39	0.39	3.125	0.195	0.39
		EA	3.125	3.125	0.39	0.098	0.78
		AC	3.125	0.78	0.39	0.098	0.098
		H2O	3.125	0.39	3.125	0.195	0.39
Peltophorum africanum Mosehla	bark	MeOH	0.098	3.125	0.39	1.56	0.195
		EA	1.56	0.195	0.195	0.78	0195
		AC	0.195	0.098	0.195	0.195	0195
		H2O	0.098	0.78	0.195	0.39	0.39
Aloe marlothii Kgokgopa ya go ema	leaf	MeOH	0.39	3.125	3.125	0.39	3.125
		EA	0.78	0.195	0.098	0.098	0.78
		AC	0.78	1.56	3.125	3.125	0.39
		H2O	0.39	0.78	0.78	0.39	1.56
Sclerocarya birrea Morula	Bark	MeOH	0.39	1.56	0.195	0.39	0.78
		EA	0.39	0.78	0195	0195	0.195
		AC	0195	0.195	0195	1.56	0.39
		H2O	1.56	0.39	0.39	0.39	1.56
Malva parviflora (L)	Leaf	MeOH	0.39	0.195	0.195	0.39	0.39
		EA	0.78	0.098	0.78	0.78	0.098
		AC	0.39	0.195	0.195	0.39	3.125

		H2O	0.39	0.39	3.125	0.39	0.195
Aloe zebrina	Leaf	MeOH	0.78	0.098	0.78	0.39	0.39
		EA	3.125	1.56	0.78	0.78	0.098
		AC	3.125	1.56	0.78	0.39	3.125
		H2O	6.25	0.39	3.125	0.39	0.195
Euphorbia tirucali Motlhoko	Leaf	MeOH	0.39	0.39	3.125	0.39	1.56
		EA	0.78	0.098	0.78	0.39	0.78
		AC	0.39	3.125	0.39	0195	0.195
		H2O	0.39	0.195	0.39	1.56	0.39
Senna italica Morotwanaditshoshi wa fase	root	MeOH	0.39	0.39	3.125	0.78	0.098
		EA	0.78	0.098	0.78	3.125	1.56
		AC	0.39	3.125	0.39	3.125	1.56
		H2O	0.39	0.195	0.39	6.25	0.39
S. brachypetala	Leaf	MeOH	0.39	0.39	3.125	0.39	3.125
		EA	0.78	0.098	0.78	0.098	0.78
		AC	0.39	3.125	0.39	3.125	0.39
		H2O	0.39	0.195	0.39	0.195	0.39
Gentamacin			0.02	0.04	0.02		
Amphotericin B						0.16	0.04

The traditional ethno-veterinary therapeutic applies are existence monitored by the rural vernacular complete which a quantity of veterinary infections are accomplished in the increasing countries. The custom of antibiotics and further chemical harvests are forbidden for animal healthcare in a quantity of countries since of human healthcare. The World Health Organization (WHO) states that 74% of the medicines derived from plant means have a modern suggestion that compares with their traditional, cultural uses [6]. Results acquired in the current study exposed that the tested plant extracts posses' probable antibacterial activity against S. aureus, E.coli, S. epidermidis and Aspergillus fumigatus (Table 1). Each plant extract of the eleven plant species were tested at three different concentrations (mg/ml) to see their inhibitory effects against bovine mastitis isolated pathogens. Of the ten candidate plants in this study, All showed significant antibacterial activity against all the tested bacteria and the remaining plants showed moderate activity after extraction.

All the tried plant extracts had MIC values ranging from 0.098 to 3.125 mg/ml against microbial strains. Out of all the tested samples the acetone extracts had better antibacterial activity with MIC values ranging from as low as 0.09 mg/ml compared to the samples extracted using traditional methods. However water extracts had the highest antifungal activity with MIC values ranging from 0.098-3,125 mg/ml compared to organic solvent extracts which had MIC values ranging from 0.098-3.125 mg/ml. Water extracts also had the highest antibacterial activity compared to acetone with MIC values ranging from 0.098 to 3.125 mg/ml. The results indicate that relatively polar compounds that are extracted by water may be responsible for the antifungal and antibacterial activity. However for antibacterial activity, S. brachypetala methano extract had the highest activity (MIC = 0.098 mg/ml). Since there are no reports yet on the biological activity of Euphorbia petricola and Aloe

zebrine (Table 2). It was very interesting that Elephantorrize elephantine, S. brachypetala Aloe marlothii, Euphorbia petricola and Cannabis Sativa extracts had better activity than the positive control amphotericin B against A. fumigatus. S.epidermidis is one of the biggest causes of nosocomial infections globally. Infections associated with S.epidermidis are serious and life threatening. The similar trend can be observed in Table 2 where acetone extracts had the highest biofilm inhibitory activity at both T0 and T24. S. brachypetala acetone extracts had highest inhibition of 93% at T0 and 64% at T24, while All extracts inhibited biofilm by more 50% at T0 and between 36-56% at T24. However, the percentage inhibition of acetone extracts decreased from T0 to T24 meaning that as the biofilm develops with time, the inhibition properties of the extracts also decreases. These properties might inhibit the S.epidermidis cells from attaching to the biofilm and prevent the biofilm from developing into a matured stage.

Table:2 Biofilm of Staphylococcus aureus

Plants (Acetone)	**Attachment %**	**Pre-Form % (24h)**
Elephantorrize elephantine(R)	77	56
Euphorbia petricola(R)	54	36
Peltophorum africanum(B)	89	48
Aloe marlothii (L)	89	52
Sclerocarya birrea(bark)	69	45
Malva parviflora (L)	74	44
Aloe zebrine	57	37
Euphorbia tirucali(L)	73	47
Cannabis Sativa(L)	55	36
Senna italica (R)	54	42
S. brachypetala	93	64
Rifampicin		

The cytotoxicity was done by means of an in vitro technique with Vero monkey kidney cells. The LC_{50} values ranged from 35.04 to 535µg/mL. Extracts of Cannabis Sativa had the maximum LC_{50} (lowest toxicity) of 535µg/mL. Euphorbia petricola had an LC_{50} of 35.04 µg/mL, which is comparatively toxic. The outcomes showed that all the herbal abstracts examined were a reduced amount of cytotoxic to Vero cells than the affirmative control, doxorubicin. Separately since Euphorbia petricola and Elephantorriza elephantina, all extracts had LC_{50} values are far more than 30 µg/mL which may indicate that they should be use more cautiously than other. In accumulation to establishing the possible care of herbal abstracts for their probable use, standard cell-based toxicity analyses are also done in vitro at an premature phase of the drug improvement method in demand to eliminate high-risk materials [25].

Table 3: Cytotoxicity of acetone extracts from nine species of the on Vero

No	**Plants Extract**	**%Inhibition**
1	**Elephantorriza elephantina**	39.6
2	**Euphorbia petricola**	35.04

3	**Peltophorum africanum**	88,27
4	**Aloe marlothi**	205
5	**Sclerocarya birrea**	326.01
6	**Malva parviflora (L)**	92.6
7	**Aloe zebrina**	78.7
8	**Euphorbia tirucali**	83.69
9	**Cannabis Sativa**	535
10	**Senna italica**	46.31
11	**S. brachypetala**	90
11	**Doxorubicin**	9,79

Furthermore, this benefits to safeguard that the biological activities of the herbal abstract is not due to a broad metabolic toxic influence. When matched to the LC50 worth of doxorubicin, the abstracts are beyond the severe boundary of the NCI and the herbal can be classified non-cytotoxic. Nevertheless, in vitro cellular toxicity might not compare to complete animal toxicity [4], henceforth the wellbeing requirements to be supplementary established by means of in vivo studies [26]. Though removal of toxic mechanisms by operation of the abstract possibly will produce additional appropriate antibacterial extracts [27].

CONCLUSION

All plants showed the utmost effective antiseptic action, signifying its possible effectiveness as a broad-spectrum antiseptic agent alongside pathogens related with bovine mastitis, at comparatively non-cytotoxic applications. Moreover, the acetone extracts of confirmed little venomousness to mammalian cells, creating them stunning contenders for probable growth into herbal harvests or for separating new pure mixtures that can help as patterns for new antimicrobial medications, which can be reasonable substitutes for dealing with bovine mastitis. Water extract have shown noteworthy results which justify their use in traditional and ethnoveterinary medicine.

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Declarations

Ethical Approval

Applicable

Consent for Publication

I consent for publication

Competing Interest

N/a

Funding

N/A

Author Contribution

Only ones author work to the study

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