Indigenous Medicinal Plants Use Sekhukhune, Limpopo, South Africa as Biofilm Inhibitors for the Mycobacterium Smegmatis

Dr. Jacobus Kori Madisha

Phytomedicine, Limpopo Education, 84 Limpopo Street, Modimolle, South Africa P.O Box 1777, Marble Hall 0450, South Africa

DOI: https://doi.org/10.51584/IJRIAS.2025.100500047

Received: 24 April 2025; Accepted: 03 May 2025; Published: 07 June 2025

ABSTRACT

The use of ordinary products to stimulate healthiness is as ancient as human sophistication. In contemporary years, the awareness of ordinary harvests derived from plants as ample foundations of naturally active complexes has determined their utilisation near the examination for novel biochemical products that can lead to further pharmaceutical formulations. Opportunistic pathogens, escalation their virulence by acquiring resistance to orthodox antimicrobials, initiating infections, particularly in immunosuppressed hosts. Ethnobotanical information on these plants was obtained. Crude acetone extracts of 11 selected medicinal plants obtained in Sekhukhune, South Africa were screened for their ability to inhibit *Mycobacterium smegmatis* and a clinical strain resistant to first-line drugs and one second-line *Rifampicin* drug microplate assay to determine the minimum inhibitory concentration (MIC). Eleven plants were gathered and their acetone extracts evaluated for anti-mycobacterial activity by the micro-delusions assay. All extracts were tested for both antibacterial activity and biofilm inhibitory activity. The greatest encouraging herbal abstracts in combating biofilm, particular their extraordinary capability to decrease it to small concentrations were the necessary oils removed from Schotia brachpetala(B), Euphorbia tirucali(L), Eucalyptus camadulensis(L), Aloe marlothii (L), Elephantorrize elephantine(R), Peltophorum africanum(B**)**

Keywords: Biofilm, Antibiotic, resistance, antibacterial, Mycobacterium, Tuberculosis

INTRODUCTION

Microbial biofilms can offer a tireless and resistant setting for hazardous microorganisms inside the figure which donates to the growth of continuing inflammatory infections [8,10]. Biofilms have been originate in several structures and matters, comprising the central ear and greater breathing strip, verbal crack, circulatory coordination, lung, gastrointestinal, colon, urogenital coordination, maxilla, and undemanding muscle abrasions [11].Biofilm development establishes an different existence in which bacteria accept a multicellular comportment that enables and/or lengthens subsistence in diverse ecological positions. [17]. Biofilms system on living and non-living shells mutually in the setting and in the healthcare situation [1]. A biofilm procedure when definite microbes (for example, some types of microorganisms) stick to the external of roughly entity in a humid location and begin to duplicate. [2]. The bacteria procedure an affection to the superficial of the entity by discharging a greasy, glue-like stuff. [14]. Four prospective enticements after the development of biofilms by microorganisms through contagion are measured: (1) fortification from detrimental environments in the swarm (2) confiscation to a nutrient-rich capacity, (3) consumption of supportive welfares, (4) biofilms customarily develop as biofilms [16].A shallow that make available humidity and nutrients is the perfect surroundings for biofilm improvement. Biofilms be able to be worthy, unscrupulous, or impartial [6]. Biofilms that are fragment of an ordinary setting are neutral, although biofilms that produce on exposed wounds subsequent contagion are detrimental. The systems of biofilm establishment is elicited and synchronised by quorum detecting, intimidating ecological settings, nutrient accessibility, hydrodynamic settings, cell-to-cell message, signalling cataracts, and tributary messengers. Integrating an basic cleanser or laundry detergent increases the efficiency of biofilm elimination paralleled to cleaning with bleach alone. [15] Decolourize castoff at concentrations appropriate for nutrients communication shells ensures have some effectiveness on thermophilic bacilli and comparable biofilms, although effectiveness may be spasmodic. [3]. When patients do existent with stubborn fever,

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

unwellness, pain and supplementary indications subsequent surgery and do not profit to antibiotic management they may have a bacterial biofilm contagion. [5]. Wounds that develop sick with biofilm may take drainage, hindered or partial restorative and an disagreeable odour. Amid numerous biofilm-associated infections and syndromes, disreputable instances comprise cystic fibrosis, otitis media, periodontitis, transmissible endocarditis [4]. Since the human perception, biofilms can be classified hooked on beneficial, neutral, and harmful. Harmful biofilms influence food security, development of plant and animal infections, and intimidate medical arenas, making it crucial to develop operative and vigorous strategies to control harmful biofilms [6,7]. Antibiotics, antiseptics, and automatic elimination means are commonly used to remove biofilms and fight off related infections. Regular biofilm disruptors such as phytochemicals, sages and nourishments identical to garlic and kale, showcase capable capabilities in dropping biofilm development and addressing associated health challenges. [8]. Research have exposed garlic to be operative in distracting biofilm. Oregano is exclusively supportive for receiving rid of undesirable pathogens after the GI strip. It's vigorous possessions, carvacrol, has been revealed to impede antibiotic resilient microorganisms, germs, vermin and moulds. In the main, a examination has reviewed the acetic acid properties, which is rich in vinegar, on the establishment of biofilm and discovered that it decreases the biofilm shaped by Staphylococcus aureus.

MATERIALS AND METHODS

Plant Material

Eleven herbal were gathered from Sekhukhune in the dumping area. Herbal was acknowledged at H.G.W.J. Schweickerdt Herbarium (PRU) at the University of Pretoria deposited a herbarium voucher specimen.

Preparation of Extracts

The plants abstracts were set with acetone (ACE) by method [19] (Eloff,1998b) and as explained previously [22] (Agidew,2022).

Microorganisms Used

The bacterial strains namely activities of the were tested against Gram-negative bacteria, as well as *Mycobacterium smegmatis* (ATCC 1441).*Bacterial* cultures were grown overnight in *Mueller Hinton (MH) broth (Sigma Aldrich, SA)* and adjusted to *McFarland standard 1.*

Chemicals

Chemicals used in the assay were obtained from Highveld Biological, Johannesburg, South Africa and Doxorubicin was acquired from Pfizer, South Africa All organic solvents were of analytical grade and obtained from Sigma-Aldrich St. Louis, MO, USA. Müller Hinton agar and broth were from Sigma-Aldrich, India.

Antimycobacterial Determination of MIC (micro dilution)

The antibacterial activity of the herb extracts was studied by means of the micro dilution bioassay as describe [19] with modification as describe by [20]. MIC of the herbal abstracts was Identify. The lower the MIC is the enhanced is the activities. Antimicrobial activity of herbal abstracts takes remained categorised as worthy (MIC< 0.1 mg/mL), reasonable (0.1 ≤ MIC ≤0.625 mg/mL) and insufficient (MIC >0.625 mg/mL) [19].

Antibiofilm development formation

The technique of [13] 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 [12].

Inhibition of pre-formed biofilm

The capability of plant abstracts to inhibit extra formation and or obliteration of cell quantity was also examined.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

The biofilm biomass was quantified by means of the changed crystal violet (CV) discolouration test [12].

Crystal violet staining assay

The method of Sandasi *et al,* 2008[13] was used for this assay, a modification of [12] Djordjevic *et al,* (2002). In brief, sterile distilled water was used to wash microtitre plates, air dehydrated and also oven dehydrated 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, incubated for 15 min and later, the plates were cleaned three intervals with disinfected purified water to get rid of unreactive stain. At this level, biofilm was detected as florid ring by the side of the wells. The quantitative 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 measured using a microplate reader (BioTek) at 590 nm. The average absorbance was determined for each sample, and their respective percentage inhibition of biofilm calculated using the formula below (Sandasi *et al*., 2008) [13]:

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

RESULTS AND DISCUSSION

Table 1

Plants	Voucher no	MIC	ATTACHMENT %	PRE-FORM % (24h)
Elephantorrize elephantine(R)	PRU0130632	0.39	75	56
Euphorbia petricola(R)	PRU0130640	6	52	36
Peltophorum africanum(B)	PRU0130633	1.6	88	48
Aloe marlothii (L)	PRU0130665	*0.16*	88	52
Sclerocarya birrea(bark)	PRU0130636	3.00	68	45
Eucalyptus camadulensis(L)	PRU0130638	*0.63*	71	44
Schinus molle(Bark) seed	PRU0130651	*12.5* 3.00	56 58	37 36
Euphorbia tirucali(L)	PRU0130664	*1.3*	72	47
Cannabis Sativa(L)	PRU0130650	2.5	53	36
Senna italica (R)	PRU0130649	2.00	52	42
Schotia brachpetala(B)	PRU234371	0.0035	79	53
Rifampicin		0.02		

Therapeutic plants assist as a possible opportunity for treatment innovation to fight infections and supplementary associated diseases. Acetone extracts Schotia brachpetala(B), Euphorbia tirucali, Eucalyptus camadulensis, Aloe marlothii (L), Elephantorrize elephantine, Peltophorum africanum(B). of had MIC value of 0.0035 mg/mL to 1.6 mg/mL against Mycobacterium smegmatis. Acetone extracts of Schotia brachpetala, Euphorbia tirucali, Eucalyptus camadulensis, Aloe marlothii, Elephantorrize elephantine, Peltophorum africanum(B). Senna italica and *Cannabis Sativa* had MIC value of 2 and 2,5 mg/mL. Acetone was the best extractant, it extracted antibacterial agents which was shown by the lowest MIC values in all selected plants. The results obtained serve as a scientific validation for the use of the plants in traditional medicine for treatment of TB and additional

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue V May 2025

respirational infection as well as their effectiveness in TB treatment innovation. Madisha [20] and Khunoana et al. [21] also reported the antibacterial activity of *A. Marlothii* and *S. brachypetala* against M.TB and *M. bovis*.

CONCLUSION

The plant species *Schotia brachpetala(B) ,Euphorbia tirucali(L), Eucalyptus camadulensis(L), Aloe marlothii (L), Elephantorrize elephantine(R), Peltophorum africanum(B)* displayed active antiseptic activity concerning *M. smegmatis* validating their promising as foundations of TB treatment clue. Henceforth, the capable recommendation amongst the supposed highest classes of complexes in the abstracts and favourable activities in the study may lead in the prospect isolation and antiseptic assessment of the bioactive compounds. Further phytochemical and pharmacological investigations of these herbal are critical and noteworthy.

DECLARATIONS

Ethical approval

N/A

Consent for publication

I am the only author

Availability of data and material

Available

Competing interest

No conflict of interest

Funding

No funding

Authors' contribution

One author work

REFERENCES

1. De La Fuente-Núñez C, Korolik V, Bains M, Nguyen U, Ebm B, Horsman S, Lewenza S, Burrows L, Rew H. Inhibition of Bacterial Biofilm Formation and Swarming Motility by A Small Synthetic Cationic Peptide. Antimicrobial Agents and Chemotherapy. 2012;56(5):2696.
2. Mm B, Rohloff J. Antibiofilm activity of essential oils and plant extracts against Staphylococcus Aureus and Escherichia Coli biofilms. Food Microbial. 2016;61,156–64.
3. Jefferson KK. What drives bacteria to produce a biofilm? FEMS Microbiol Lett 2004;236(2):163–73.
4. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010;8(9):623–33.
5. Hu W, Li L, Sharma S, Wang J, McHardy I, Lux R. DNA builds and strengthensthe extracellular matrix in Myxococcus xanthus biofilms by interacting with exopolysaccharides. PLoS One 2012;7(12):e51905.
6. Kim H, Park H. Ginger extract inhibits biofilm formation by Pseudomonas aeruginosa PA14. PLoS One 2013;8(9):e76106.
7. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010;35(4):322–32.
8. Donlan RM, "Role of biofilms in antimicrobial resistance," ASAIO Journal, vol. 46, no. 6, pp. S47–S52, 2000.

9. Liu Y, Shi L, Su L, Mei HC, Van Der JPC, Ren Y. Nanotechnology-based antimicrobials and delivery systems for biofilm infection control Yong. Chem. Soc. Rev. 2019;428–446. doi: 10.1039/c7cs00807d.
10. Jamal M, Ahmad W, Andleeb S et al., "Bacterial biofilm and associated infections," Journal of the Chinese Medical Association, vol. 81, no. 1, pp. 7–11, 2018.
11. Donlan RM, "Biofilm formation: a clinically relevant microbiological process," Clinical Infectious Diseases, vol. 33, no. 8, pp. 1387–1392, 2001.
12. Djordjevic D, Wiedmann M, Mclandsborough L. Microtiter plate assay for assessment of Listeria Monocytogenes biofilm formation. Appl Environ Microbiol. 2002;68(6):2950–8.
13. Sandasi M, Leonard CM, Viljoen AM.The effect of five common essential oil components on Listeria monocytogenes biofilms. Food Control, 2008;19,1070–1075.
14. Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Ma N, Hussain T, Ali M, Rafiq M, Ma K. Bacterial biofilm and associated infections. J Chin Med Assoc. 2018;81(1):7–11.
15. Ks L, Lou Z, Letsididi R, Mohammed K, Bl M. Antimicrobial and Antibiofilm effects of trans-Cinnamic acid Nanoemulsion and its potential application on lettuce. Lwt. 2018;94,25–32.
16. Lebeaux D, Ghigo J-M, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev. 2014;78(3):510–43.
17. Wolcott R, Costerton JW, Raoult D, and Cutler SJ, ")epolymicrobial nature of biofilm infection," Clinical Microbiology and Infection, vol. 19, no. 2, pp. 107–112, 2013.
18. ELOFF, J. N. 1998b. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica, 64**,** 711-713.
19. Madisha JK. Antimycobacterial Activities of Selected Plants Used in the Management of Tuberculosis in Sekhukhune (Limpopo Province), South Africa. MSc Dissertation. Bloemfontein: University of the Free State; 2017
20. Khunoana ET, Madikizela B, Erhabor J, Nkadimeng SM, Arnot LF, Van Wyk I, McGaw LJ. A survey of plants used to treat livestock diseases in the Mnisi community, Mpumalanga, South Africa, and investigation of their antimicrobial activity. South African Journal of Botany. 2019;126, 21-29.
21. Agidew MG.2022.Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. Bull Natl Res Cent 46, 87 .