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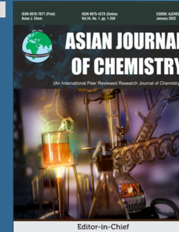

Metabolite Profiling and Antimicrobial Activity of *Aloe greatheadii* var. Davyana against Bacterial Illness

DENGA NTHAI, VUYISILE S. THIBANE and SECHENE S. GOLOLO*

Department of Department of Biochemistry and Biotechnology, Sefako Makgatho Health Sciences University, P.O. Box 235, Medunsa 0204, Ga-Rankuwa, South Africa

*Corresponding author: Tel: +27 521 4372; E-mail: Stanley.gololo@smu.ac.za

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The aim of the study was to profile the metabolite content of *Aloe greatheadii* and investigate their antibacterial potential against illness causing bacteria. The metabolite profile of *A. greatheadii* was determined and the antibacterial activity tested against Gram-positive and Gram-negative bacteria. The metabolite profile resolved on TLC plates indicated an almost similar pattern of bands for majority of the separated compounds. The results on the maximum absorption wavelength of extracts from all locations ranged between 240 and 320 nm. The most susceptible bacteria for the treatment by samples from all the provinces was the Gram-positive *E. faecalis* with MIC values ranging from 0.54 to 0.067 mg/mL. It was interesting to note that susceptibility of the tested bacteria to treatment by the plant extracts was not specific to either Gram-positive or Gram-negative bacteria.

Keywords: *Aloe greatheadii*, Antibacterial activity, Commensal bacteria, Natural products.

INTRODUCTION

The Aloe specie, *Aloe greatheadii* (spotted Aloe), belongs to the Asphodelaceae family and is mostly encountered in the Grassland and Bushveld biomes and it often forms extensive stands in overgrazed areas [1]. *Aloe greatheadii* is a beautiful garden plant, which is stemless and grows singularly or in groups. The succulent leaves are arranged in a basal rosette with leaves lance-shaped and spots arranged in distinct bands that are usually with a whitish green spots and margins armed with sharp, dark brown teeth [2]. The plant is one of the most widely distributed Aloe specie and grows wild in the northern parts of South Africa [3]. The plant is used in traditional medicine for treatment of bacterial infections, urinary tract infections, treatment of arthritis, skin cancer, burns, eczema, psoriasis, diabetes and high blood pressure [4]. Medicinal plants have been reported to express a wide range of antimicrobial activities with several proposed modes of action [5].

Many bacterial cultures are known to be commensals in the human body, however reports have shown that under stress conditions for the immune system this bacteria can cause illness and disease [6]. Bacteria belonging to both Gram-positive and Gram-negative groups have been reported in causing illness and disease in the human body. The Gram-negative bacterium, *Escherichia coli* and *Pseudomonas aeruginosa*, are commonly found in the gastrointestinal tract. These bacterial cultures are known to cause respiratory related illness, neonatal infections related to pneumonia and urinary tract infections in immune compromized systems [7,8]. The Gram-positive bacteria *S. aureus* is a notorious bacterium in hospitals and a leading cause of nosocomial infections just like Gram-negative *P. aeruginosa*. The bacterial cultures, *S. aureus* and *E. faecalis*, are commensals on healthy skins and have been reported to cause wound infections and skin infections in humans [9,10]. The widely published bacterial resistance of many bacterial cultures to antibiotic treatment necessitates continued exploration on natural products with antibacterial potential. Therefore, this study was aimed at profiling the metabolite content of *A. greatheadii* from four South African provinces and investigate their antibacterial potential against illness causing bacteria.

EXPERIMENTAL

Plant extract preparation: Leaves of *Aloe greatheadii* var. davyana were collected in the wild from different locations each in Limpopo, Mpumalanga, Gauteng and North-West

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provinces of South Africa using a convenient sampling method. Plants were identified by Dr. Bronwyn Egan, Department of Botany, University of Limpopo (UL) and a voucher specimen (UNIN 12990) was prepared and deposited in the Leach Herbarium (UL). Leaves were washed with sterile distilled water (H_2O) to remove excess dirt. After washing, the leaves were firstly chopped into smaller pieces, dried at room temperature until constant dry weight and finally ground into powder using a table top blender. The ground plant materials (5 g) were extracted with hexane, acetone and methanol using cold maceration with agitation for 24 h at room temperature. The solution was filtered using Whatman No.1 filter paper and allowed to dry under a stream of air. Extracts were kept in a tightly sealed container and stored in the dark until analysis.

Thin layer chromatography (TLC) analysis: The metabolite profile of *A. greatheadii* was resolved by the TLC chromatographic technique, separating non-volatile metabolites on a sheet of aluminium foil coated with adsorbent silica gel material [11]. Aliquots (1 mg/mL) of extracts were loaded on TLC (silica gel 60 F_{245}, 20 cm × 20 cm; Merck, South Africa) plates and resolved using three different mobile phases of hexane:ethyl acetate (9:1 v/v); chloroform:methanol (9:1 v/v) and ethyl acetate:methanol:water (8:4:1 v/v/v) at a distance of 1 cm from the lower edge and 1 cm between each band. Chromatograms were developed in closed tank with atmosphere saturated with eluent vapour. The plates were dried overnight or until there were no traces of solvent smell, at room temperature under a stream of air to remove excess solvent. The dried plates were sprayed with a vanillin-sulphuric solution (0.1 g vanillin, 28 mL methanol, 1 mL sulphuric acid) and placed in an oven at 105 ºC for 5 min for optimal colour development. Compound were also visualized under UV-light and the visible bands were then used to calculate retention factor (R_f) values and compared between different provinces.

Ultraviolet visible (UV-VIS) light spectrophotometry analysis: The measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface was conducted on methanol extracts [12]. Briefly, plant extracts were re-dissolved in dimethyl sulfoxide (DMSO) to constitute 10 mg/mL of the extracts and diluted ten-fold. UV-Vis spectral profiles of the diluted extracts were obtained using a CECIL 1021 spectrophotometer (Labotec, South Africa). Wavelengths of maximum absorbance were recorded. The assay was performed in duplicates and repeated three times.

Antimicrobial activity assay: The antibacterial activity of the plant extracts was evaluated using the micro-dilution bioassay to determine the minimum inhibitory concentration (MIC) values against the Gram-positive (*S. aureus* and *E. faecalis*) and Gram-negative (*P. aeruginosa* and *E. coli*) as described by Eloff [13]. Plants extracts were re-suspended in 10% DMSO. Bacterial cultures were grown overnight at 37 ºC in Mueller-Hinton (MH) broth and a bacterial inoculum of 1×10^6 colony forming units/mL, actively growing bacteria, was prepared. Briefly, a volume of 150 µL at an extracts concentration of 10 mg/mL was added in the first well of the plate and subsequently diluted down with 150 µL of sterile distilled water to prepare different extract concentrations. After dilution, 50 µL of actively growing bacterial inoculum was added into each well and the plate incubated in a humidified environment at 37 °C for 24 h. After incubation, 50 µL (0.2 mg/mL) of the growth indicator *p*-iodonitrotetrazolium chloride (INT) was added in each well and the plate further incubated at 37 ºC for 2 h. A gentamycin solution (0.1%) was used as a positive control and 10% DMSO used as a negative control. The MIC was assayed as the lowest concentration that inhibited bacterial growth and remained colourless. The assay was performed in duplicates and repeated three times.

Data analysis: Data obtained was presented in the form of tables and histograms as mean ± standard deviation of triplicates. Statistical analysis amongst the results of extracts of the plant samples from different provinces was done by one-way ANOVA using SPSS version 18 statistical package. Differences between groups were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

TLC assay: Plant extracts of *A. greatheadii* from four different provinces, Limpopo, Mpumalanga, Gauteng and North West, were subjected to TLC analysis to profile their metabolite content. The TLC plate method enables the separation of phytoconstituents within the extracts based on their polarities due to the interaction of the stationary and mobile phases [14]. The results for the TLC analysis are expressed as bands of metabolites indicating their respective R_f values in Fig. 1a-c. The metabolite profile of the hexane extracts from Limpopo, Mpumalanga, Gauteng and North-West eluted with the non-polar solvent solution of hexane:ethyl acetate (HE 9:1) is expressed in Fig. 1a. The results indicate an almost similar pattern of bands and metabolite profile for majority of the compounds separated. The extracts from Mpumalanga expressed a noticeable absence of metabolites with a R_f values of 0.23, 0.54 and 0.77, which were present in samples from the other provinces. The other notable difference was the absence of a metabolite with a R_f value of 0.48 for extracts from Gauteng that is present in all the other samples. The metabolite profile for acetone extracts from Limpopo, Mpumalanga, Gauteng and North-West eluted with the intermediate solvent solution of chloroform:methanol (CM 9:1) is expressed in Fig. 1b. The metabolite profile was significantly not different between the four provinces. One of the notable difference in the metabolite profile was the presence of a compound with a R_f value of 0.51 for a sample from Gauteng province which was not detected in the other province sample. Compounds with a R_f value of 0.18, 0.30 and 0.65 were detected only in extracts from the Mpumalanga and North-West provinces. The variation in the metabolite profile of the extracts can be partly attributed to the differences of biomes that exist between the four different provinces. The metabolite profile for methanol extracts from Limpopo, Mpumalanga, Gauteng and North-West eluted with the polar solvent solution of ethyl acetate:methanol:water (EMW 8:4:1) is expressed in Fig. 1c. Compound with a R_f value of 0.87 was detected only in samples from Limpopo and Gauteng provinces, while a compound with an R_f value of

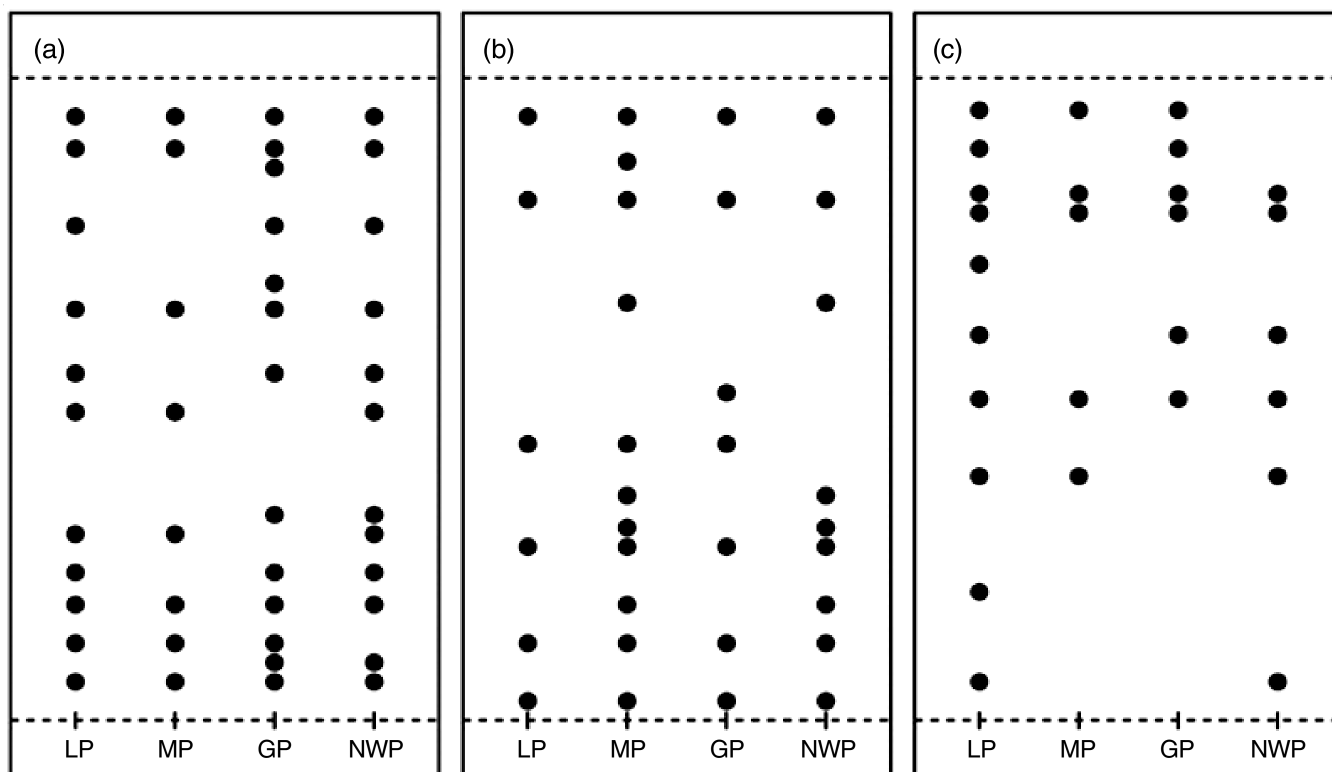

Fig. 1. TLC chromatogram of *Aloe greatheadii* showing separated metabolites from Limpopo Province (LP), Mpumalanga Province (MP), Gauteng Province (GP) and North West Province (NWP). (a) Metabolites extracted with hexane ran with a non-polar mobile phase of hexane:ethyl-acetate (HE) (9:1). (b) Metabolites extracted with acetone ran with intermediate mobile phase of chloroform:methanol (CM) (9:1). (c) Metabolites extracted with methanol ran with polar mobile phase of ethyl-acetate:ethanol:water (EEW): (8:4:1)

0.25 was only detected in a sample from the North-West. The influence of the plants genotype and adaptation to environmental stress has been frequently reported to contributing in the differences in the metabolite profile of plants [15,16].

Ultraviolet-visible light spectroscopy: The measurement of the absorbance of an extract solution at specific wavelengths can be a useful tool in determining the concentration of the solution when applying the Beer-Lambert's law [12]. The UV-vis light spectrophotometric analysis on the other hand makes it possible to determine the nature of present phytochemicals within plant extracts based on their maximum absorption wavelengths peaks derived from their absorption spectra. This quantitative and qualitative analysis of metabolites in an extract solution can be very helpful when looking to determine the bioactivity of the plant. Different groups of phytochemicals absorb light at different wavelengths of the spectrum which, enables determination of the nature of phytochemicals [17]. The absorption peaks of the extracts from the different location was similar, with their levels being the only difference. Fig. 2 represents the absorbance peaks, with the different provinces represented as Limpopo (A), Mpumalanga (B), Gauteng (C) and North West (D). Phenolic compounds are the most abundant plant secondary metabolites with maximum absorption reported to peak in the range of 210-290 nm and 305-390 nm [17]. Absorption peaks are directly proportional to the presence of functional groups and the double bonds added to a conjugated system. The predominantly non-polar metabolites extracted with hexane expressed a maximum absorption peak at a wavelength of 280 nm in samples from Limpopo (Fig. 2a), Mpumalanga (Fig. 2b) and Gauteng (Fig. 2c) provinces. There was a recorded difference in the absorption spectrum for extracts from Limpopo and Mpumalanga when compared to extracts from Gauteng and North West provinces in samples extracted with acetone. The maximum absorption peak was recorded at a wavelength of 240 nm and 320 nm for samples from Limpopo and Mpumalanga provinces. Samples extracted with methanol expressed a similarly absorption spectrum for extracts from Limpopo and Gauteng provinces when compared to extracts from Mpumalanga and North West provinces. The maximum absorption peak was recorded at a wavelength of 240 nm and 320 nm for samples from Limpopo and Gauteng. The results for the study indicate that the maximum absorption wavelengths peaks of the extracts from all four locations range between 240 nm and 320 nm. Metabolites that are known to peak at this wavelength includes; hydroxycinnamic acids and their derivatives, apigenin, luteolin, flavonol 3-*O*-glycosides and kaempferol [17]. Hydroxycinnamic acids (caffeic acid, ferulic acid and *p*-coumaric acid) have been reported to be effective in inhibiting the growth of *E. coli* and *S. aureus* through the disruption of bacterial cell membrane and consequently leading to cellular content leakage [18,19]. Therefore, the notable antibacterial activity of *A. greatheadii* might be as a result of hydroxycinnamic acids and their consequence disruption of bacterial cell wall. The most notable difference is the variation in the level of the phenolic compounds between the different locations [20]. Interspecies variation and varying geographic

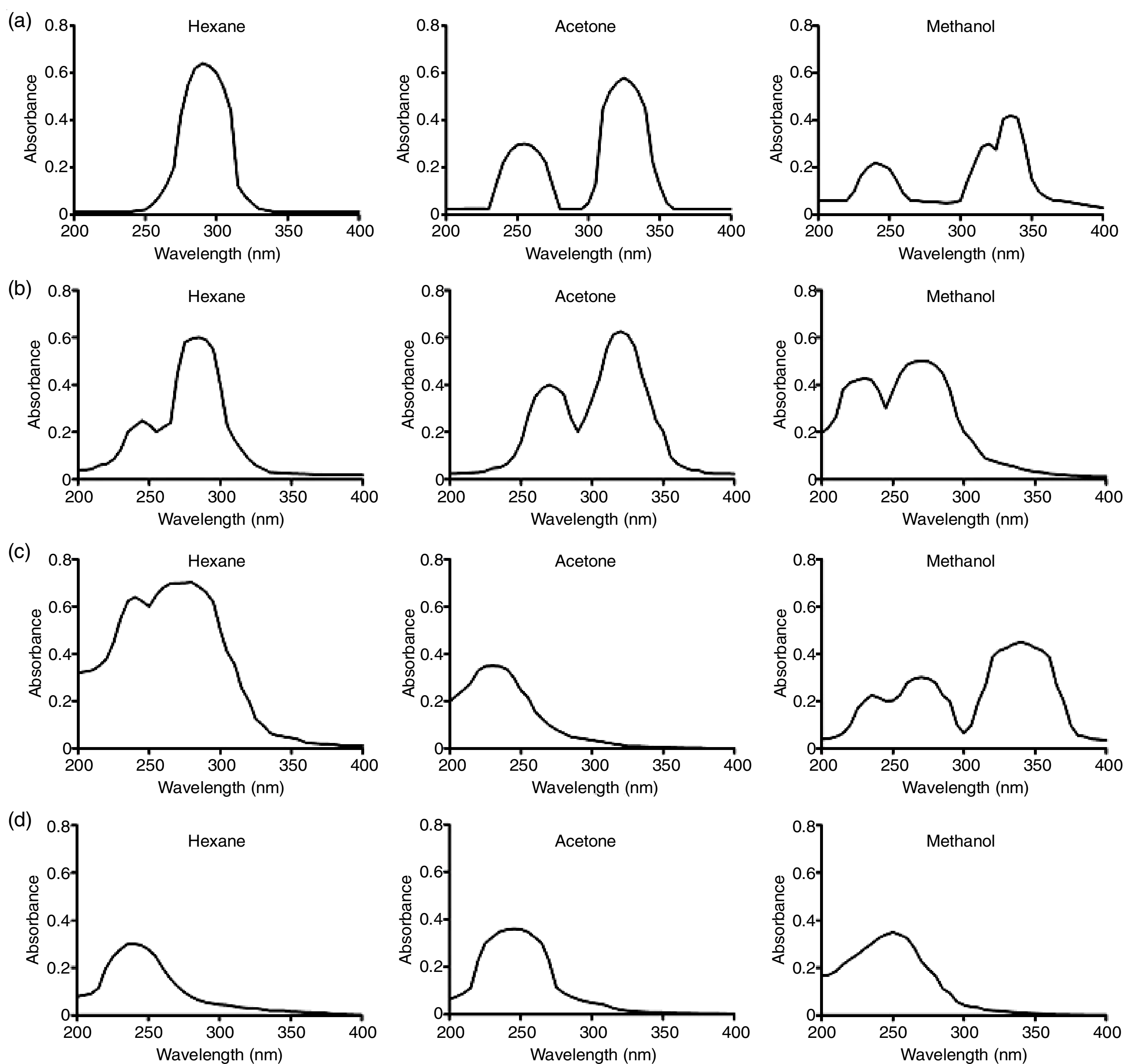

Fig. 2. UV-vis light spectrophotogram expressing absorbance peaks of leaves extracts of *Aloe greatheadii* from Limpopo (a), Mpumalanga (b), Gauteng (c) and North West (d) provinces extracted with hexane, acetone and methanol

location and soil conditions has been reported to affect the level and content of phytochemical compounds in plants [21,22].

Antimicrobial activity assay: The results of the antibacterial activity of leave extracts of *A. greatheadii* from the four provinces in South Africa are presented in Table-1. Extracts of leaves of *A. greatheadii* with different polarities as extracted with hexane, acetone and methanol exhibited varying antibacterial activity against the illness causing bacterial cultures. The non-polar extracts of hexane from all the provinces were the least active extract against all the tested bacteria. The only notable antibacterial activity for hexane extracts recorded was for the Gauteng sample against the Gram-negative *E. coli*. The most susceptible bacteria to the treatment by samples from all the provinces was the Gram-positive *E. faecalis* with MIC values ranging from 0.54 to 0.067 mg/mL. Susceptibility of Gram-positive bacteria, when compared to Gram-negative bacteria, has mainly been attributed to their lack of the an outer membrane. The outer membrane lipid bilayer of bacteria plays a critical role as a barrier function against antibiotics penetrating into the bacterial inner cell membrane [23,24]. Furthermore, the methanol extracts from samples from Limpopo province were effective at inhibiting all the tested bacterial cultures with MIC values ranging from 0.27 to 0.13 mg/mL. Methanol, a polar solvent, has a significantly high solvent strength value and potential of extracting a wide array of metabolites [25]. Furthermore, methanol has been reported to be efficient in screening and extracting phytoconstituents with antimicrobial activity from plant material [13]. The methanol extracts from

TABLE-1
MIC VALUES (mg/mL) OF *Aloe greatheadii* LEAVES EXTRACTS FROM FOUR SOUTH AFRICAN PROVINCES AGAINST *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* AND *Enterococcus faecalis*

Location/Province	Solvent	MIC values (mg/mL)			
		P. aeruginosa	*E. coli*	*S. aureus*	*E. faecalis*
Limpopo	Hexane	2.14	1.07	2.14	2.14
	Acetone	0.54	1.07	4.28	0.54
	Methanol	0.27	0.27	0.27	0.13
Mpumalanga	Hexane	2.14	1.07	2.14	2.14
	Acetone	2.14	1.07	0.54	0.13
	Methanol	4.28	0.54	0.54	0.13
Gauteng	Hexane	2.14	0.54	1.07	2.14
	Acetone	2.14	1.07	1.07	0.13
	Methanol	1.07	0.13	1.07	0.067
North West	Hexane	2.14	1.07	4.28	2.14
	Acetone	0.27	0.27	2.14	1.07
	Methanol	0.13	0.13	1.07	0.54

Note: Extract highlighted in bold expressed good antibacterial activity with MIC values less than 1 mg/mL

North-West sample expressed antibacterial activity against the Gram-positive (*P. aeruginosa*) and Gram-negative (*E. coli* and *E. faecalis*) with MIC values of 0.13, 0.13 and 0.54 mg/mL, respectively. The methanolic extracts from Mpumalanga sample also expressed antibacterial activity against the Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *E. faecalis*) with MIC values of 0.54, 0.54 and 0.13 mg/mL, respectively. The anti-bacterial activity of methanol extracts of Gauteng sample was only recorded against the Gram-negative (*E. coli* and *E. faecalis*) with MIC values of 0.13 and 0.067 mg/mL, respectively. Acetone extracts were the second most active extracts with antibacterial activity against a number of the tested microorganisms. Acetone extracts of the Limpopo sample were active against the Gram-positive (*P. aeruginosa*) and Gram-negative (*E. faecalis*) with MIC values of 0.54 mg/mL against both bacteria. Extracts of the North West sample also demonstrated antibacterial activity against the Gram-positive (*P. aeruginosa*) and Gram-negative (*E. coli*) with MIC values of 0.27 mg/mL against both bacteria. However, the samples collected from Mpumalanga and Gauteng could only inhibit the Gram-positive (*S. aureus*) and Gram-negative (*E. faecalis*) with MIC values of 0.54 and 0.13 mg/mL, respectively. It was interesting to note that suscep-tibility of the tested bacteria to treatment by the plant extracts was not specific to either Gram-positive or Gram-negative bacteria. It appears the nature of the phytoconstituents present in the plant extract was the major determinant on the antibacterial activity of the plants. The notable variation in the content of phytochemical compounds as expressed by the results for the TLC and UV-Vis light spectroscopic analyses can be attributed to interspecies variation and varying geographic location [22]. Furthermore, the antibacterial activity of *A. greatheadii* has been shown to be as a result of the nature of phytochemical compounds present in the plant with several secondary metabolites identified as major components in *A. greatheadii* [26].

Conclusion

The antibacterial activity of *Aloe greatheadii* has been shown to be as a result of phytochemical compounds present in the plant that differs with the geographical location of growth. The notable antibacterial activity could be attributed to the deductible presence of compounds such as hydroxycinnamic acids in extracts of *A. greatheadii*. The phytoconstituents from the plant have demonstrated potential in combating illness-causing bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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