Scientific rationale for traditional use of plants to treat tuberculosis in the eastern region of the OR Tambo district, South Africa

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Abstract

Ethnopharmacological relevance: Tuberculosis (TB) remains a major health problem for humans worldwide, and was responsible for 1.4 million human deaths in 2015 alone. Although there is treatment for TB, emerging multi-drug, extensively drug and totally drug resistant forms of this disease, as well as co-infection with human immunodefiency virus (HIV) continue to worsen the situation. South Africa is among countries with reported traditional use and published documentation of such knowledge concerning the use of plants against TB. Based on a previous study where plants used traditionally for treating TB in the eastern region of OR Tambo district, South Africa, were documented, the present study aimed to determine the antimycobacterial effect, cytotoxicity and genotoxicity of plants selected from that list.

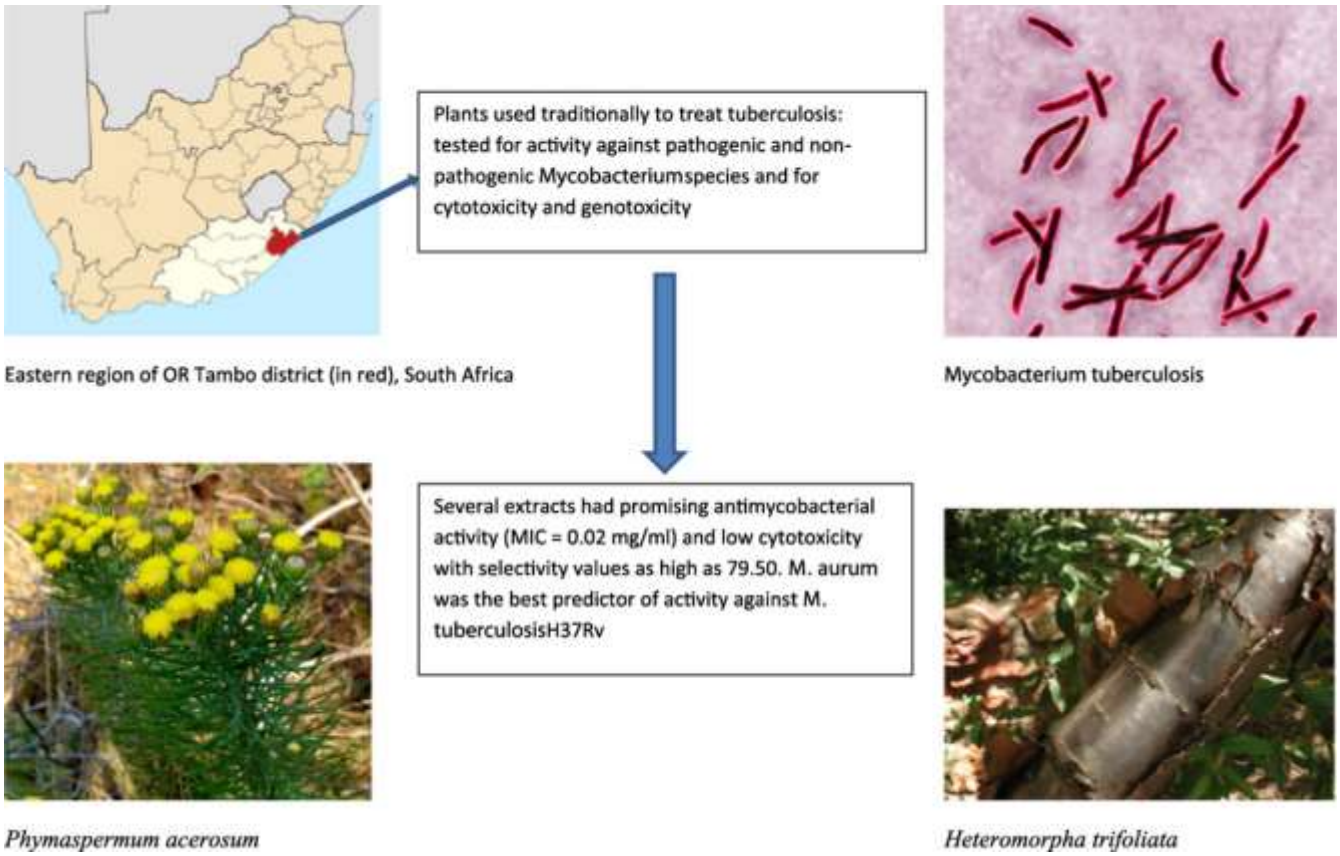
Material and methods: Acetone, 70% ethanol, cold and hot water extracts were tested for antimycobacterial activity against saprophytic *Mycobacterium* species including *M. aurum*, *M. bovis* BCG, *M. gordonae*, *M. fortuitum*, and *M. smegmatis* using a microdilution method. Extracts with MIC values less than 1 mg/ml against at least three *Mycobacterium* strains were evaluated for antimycobacterial activity against pathogenic *Mycobacterium* strains including *M. tuberculosis* H37RV, *M. tuberculosis* and *M. bovis*, cytotoxicity (against Vero monkey kidney and bovine dermis cells), and genotoxicity (against *Salmonella typhimurium* TA98 and TA100).

Results: The MIC values of the extracts ranged from 0.02-2.50 mg/ml, LC_{50} values of twenty-nine extracts ranged from 0.001- >1 mg/ml and the highest selectivity index was 79.50, an

extremely promising value. *Phymaspermum acerosum* roots and leaves (ethanol and water) extracts had the best MIC value (0.02 mg/ml) against at least one *Mycobacterium* strain. It was interesting to note the lack of cytotoxicity of these extracts with the highest selectivity index value of 39.75. *Pterocelastrus echinatus* bark acetone and *Protorhus longifolia* leaf hot water extracts were the most cytotoxic with LC_{50} = 0.001 and 0.008 mg/ml, respectively, whereas *Pittosporum viridiflorum* bark water extract was genotoxic. Among non-pathogenic strains, *M. gordonae*, *M. aurum*, *M. smegmatis* showed good correlation of activity with pathogenic *M. tuberculosis* H37Rv, *M. tuberculosis* and *M. bovis*, however, *M. aurum* was the best predictor with *Mycobacterium tuberculosis* H37Rv (correlation coefficient value=0.73)

Conclusion: The results indicate that *Heteromorpha trifoliata* and *Phymaspermum acerosum* should be investigated further for antimycobacterial efficacy against drug resistant strains of *Mycobacterium*.

Graphical abstract



Keywords: Antimycobacterial, OR Tambo district, *Phymaspermum acerosum*, *Heteromorpha trifoliata*, safety evaluation, cytotoxicity

1. Introduction

Tuberculosis (TB), an old yet re-emerging bacterial infectious disease, is one of the leading ten causes of mortality and morbidity worldwide infecting both humans and animals (cattle, and several other domestic and wild animals) (Fyhrquist et al., 2014; WHO, 2017). This disease is highly contagious, transmitted from one individual to another via droplet nuclei from a person with an active TB infection. TB is caused by organisms belonging to the *Mycobacterium tuberculosis* complex which includes *M. africanum*, *M. bovis*, the attenuated *M. bovis* Bacille Calmette–Guerin (BCG) vaccine strain, *M. canetti*, *M. caprae*, *M. microti*, *M. pinnipedii*, and *M. mungi*. There is treatment for TB, but it has limitations. The treatment protocol for TB is lengthy (6 months or longer for more resistant strains), the drugs have side effects, and poor compliance of patients infected with this disease has led to development of multi drug resistant (MDR), extensively drug resistant (XDR) and totally drug resistant (TDR) TB strains. Additionally, the association of TB with human immunodefiency virus (HIV) has worsened the complication of TB therapy. In 2015, there were an estimated 10.4 million new cases of TB worldwide, and people living with HIV accounted for 1.2 million of these (WHO, 2017). South Africa is among six countries accounting for 60% of new TB cases in 2015 (WHO, 2017). The number of TB deaths fell by 22% between 2000 and 2015; however, the disease still claimed approximately 1.4 million lives in 2015 alone, and 0.4 million were HIV positive people (WHO, 2017).

Many efforts have been made worldwide to discover and develop new anti-TB drugs that are superior to those available today in terms of their bactericidal activity, efficacy against drug resistant TB strains, safety tolerability, and suitability for coadministration with antiretroviral drugs (ARVs). Four compounds, namely moxifloxacin, gatifloxacin, bedaquiline, and delamanid have been discovered by the current global TB drug development programme, and these are still under evaluation for phase III clinical trials (Mdluli et al., 2015). Although bedaquiline or sirturo was approved by the United States Food and Drug Administration (FDA) as part of combination therapy for treatment of adults with MDR TB (Mahajan, 2013), the drug's potential risks, which include death, have raised concerns, and this has resulted in the drug being mandated for use only when there are no other alternative treatment options available (Mahajan, 2013).

Globally, plants have been reported for traditional use as medicine since antiquity for treatment of various diseases including TB. In South Africa, several ethnobotanical surveys

documenting the traditional use of plants against TB have been conducted. These include surveys done in Limpopo, Free State, and Eastern Cape (Semenya and Maroyi, 2013; Phungula et al., 2014; Famewo et al., 2017, Madikizela et al., 2017). Worldwide, there are many reports of primary screenings of crude plant extracts by researchers with the intention of identifying new anti-TB compounds (Cantrell et al., 1998; Molina-Salinas et al., 2006; Green et al., 2010; Luo et al., 2011, Masoko and Nxumalo, 2013; Nguta et al., 2016). The influence of plant use based traditional medicine on drug discovery has been profound, with several clinically active drugs derived from them. As a result, structurally diverse compounds with anti-TB activity have been isolated from plants showing potential against TB causative bacterial strains, and several of them have shown significant antimycobacterial activities (Ramos et al., 2008, Fyhrquist et al., 2014). However, more plants still need to be investigated for their efficacy. In 2008 a review on the antimycobacterial potential of South African medicinal plants highlighted that close to 180 plants are used in local traditional medicine but only about 30% have been tested for antimycobacterial activity, largely only against non-pathogenic mycobacteria (McGaw et al., 2008a).

Further recent work has identified more plant species used to treat TB in southern Africa. In our previous research, plants used in the eastern region of OR Tambo district, Eastern Cape province, South Africa, for traditionally treating TB were documented (Madikizela et al., 2017). In the present study ten plants from that list were selected for biological analysis. The selection of the plants used in this study followed scrutiny of available literature for reported antimycobacterial studies and discovering that such information was lacking. Therefore, the aim of this study was to determine the antimycobacterial effect of ten selected plants used in the eastern region of OR Tambo district in traditional anti-TB therapy. Since most in vitro antimycobacterial studies have been conducted against non-pathogenic, saprophytic *Mycobacterium* species and studies reporting correlations between activity of such species and pathogenic species are sparse and contradictory, it is useful to compare activities of the extracts against different *Mycobacterium* species to determine which non-pathogenic *Mycobacterium* species correlates best to the pathogenic *M. tuberculosis* in terms of susceptibility to plant extracts. More information is required to support the use of preliminary antimycobacterial screening methods using fast-growing saprophytic mycobacteria, or slower-growing species with lower safety concerns such as *M. bovis* BCG (McGaw et al., 2008b).

The World Health Organisation (WHO) continues to encourage the use of medicinal plants to supplement their health care program, provided that they are proven to be safe (Hong and Lyu, 2011). Worldwide, the potential toxicity of some of the more popularly used herbal remedies is a cause for concern, thus, it is necessary to assess their toxicity to ensure their relative safety as it would be dangerous to assume that all plant extracts are safe to use. Therefore, this study further aimed to determine *in vitro* if extracts from selected medicinal plants were cytotoxic or genotoxic.

2. Materials and methods

2.1 Plant collection and extraction

Specimens of ten selected plants; *Gerrardina foliosa* Oliv., *Haemanthus albiflos* Jacq, *Heteromorpha trifoliata* (H.L. Wendl.) Eckl. & Zeyh., *Hypoxis colchicifolia* Baker, *Pachycarpus concolor* E. Mey., *Phymaspermum acerosum (*DC.) Källersjö, *Pittosporum viridiflorum* Sims, *Protorhus longifolia* (Bernh.) Engl, *Pterocelastrus echinatus* N.E. Br., and *Talinum caffrum* (Thunb.) Eckl. & Zeyh. were collected from different provinces in South Africa (Gauteng, Mpumalanga, KwaZulu-Natal and Eastern Cape), and identified at the HGWJ Schweickerdt Herbarium, University of Pretoria, South Africa. Dried, ground plant material (2 g) was extracted non-sequentially with 20 ml of 100% acetone, 70% ethanol, and cold water through sonication for 1 hr. The decoction (referred to as the hot water extract) was prepared by extracting 2 g of dried material in 30 ml of distilled water and boiling for 5 minutes, cooled for 1 h at room temperature, and then filtered. All the extracts were filtered through Whatman No 1. filter paper, and the filtrates were concentrated using a rotary evaporator and stored at 8ºC until use.

2.2 Antimycobacterial assay

The Department of Agriculture, Forestry and Fisheries of South Africa granted approval under Section 20 of the Animal Diseases Act to conduct this research. The WHO (2012) biosafety standards for handling of pathogenic *Mycobacterium* strains were followed, and they included working in a Biosafety Level 2 Laboratory, wearing a protective gear, following the required procedure for decontamination and disposal of waste. All the experiments were performed in the laboratory approved by the Institutional Biosafety

Committee for Mycobacterial Cultures by University of Pretoria, in the Department of Veterinary Tropical Diseases.

Five non-pathogenic *Mycobacterium* strains, three from the American Type Culture Collection (ATCC), one from the National Collection of Type Cultures (NCTC), and one Pasteur strain; fast growing {*M. fortuitum* (ATCC 6841), and *M. smegmatis* (ATCC 1441)}, and slow growing {*M. bovis* BCG P1172, *M. gordonae* (ATCC 14470), and *M. aurum* (NCTC 10437)} were used for the antimycobacterial assay against all extracts prepared in this study. The *Mycobacterium* cultures were maintained on Middlebrook agar supplemented with 10% (v/v) oleic acid, albumin, dextrose and catalase (OADC) and glycerol, and were cultured on glycerol and OADC-supplemented Middlebrook broth. The cultures were incubated at 37^{o}C for 24-48 h for *M. fortuitum* and *M. smegmatis*, 3-4 days for *M. aurum*, and 14-21 days for *M. bovis* BCG and *M. gordonae*. Three pathogenic *Mycobacterium* strains were used to screen the antimycobacterial activity of extracts that showed MIC values less than 1 mg/ml against at least 3 non-pathogenic *Mycobacterium* strains. The pathogenic *M. tuberculosis* H37RV ATCC 27294 and *M. tuberculosis* ATCC 25177 were maintained on Lowenstein-Jensen (LJ) slants supplemented with glycerol, or pyruvate in the case of *M. bovis* ATCC 27290, for a month, and colonies were transferred into 3 ml of supplemented Middlebrook broth, homogenized by vortexing, then the larger particles were allowed to settle. All the test inoculum were prepared in supplemented Middlebrook broth, and adjusted to McFarland standard 1 equivalent to 3.0 x 10^{8} diluted to a final density of 5×10^{5} CFU/ml in the medium.

A microdilution assay according to Eloff (1998) and Jadaun et al. (2007) in a 96 well microtitre plate was used to determine the MIC values of fifty-eight extracts from the ten plants. Extracts (10 mg/ml) prepared in 10% dimethylsulfoxide (DMSO) and water (for water extracts) were serially diluted twofold (100 µl) with OADC-supplemented Middlebrook 7H9 broth down the wells of a 96 well microtitre plate. Isoniazid, streptomycin and rifampicin were used as positive controls, whereas 10% DMSO, water, inoculum and OADC-supplemented Middlebrook 7H9 broth were negative controls. Then, 100 µl of mycobacterial cultures were added in all the wells. The plates were sealed with parafilm and incubated for 24-48 h (*M. smegmatis* and *M. fortuitum*), 3-4 days (*M. aurum*) 7-10 days (*M. gordonae* and *M. bovis*, *M. bovis* BCG, *M. tuberculosis* H37RV and *M. tuberculosis*) at 37^{o}C. After the incubation period, MIC values were determined by adding 40 µl of 0.2 mg/ml of freshly prepared *p*-iodonitro-tetrazolium chloride (INT) solution. Colour detection after the addition

of INT was read as soon as colour became visible in the untreated control wells. MIC values were read as the concentrations where a marked reduction in colour formation corresponding to inhibition of mycobacterial growth was noted. Concentrations were tested in triplicate and the experiments were repeated twice. Correlations between the MIC values of the various *Mycobacterium* strains used and the pathogenic ATCC strain of *M. tuberculosis* were calculated using Microsoft Excel 2010 software (Kabongo-Kayoka et al., 2016).

2.3 Cytotoxicity assay

The cytotoxicity of plant extracts was determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay according to Mosmann (1983). Extracts with antimycobacterial activity against at least three *Mycobacterium* strains were evaluated for cytotoxicity against the African green monkey kidney (Vero) and bovine dermis cells obtained from the Department of Veterinary Tropical Diseases (University of Pretoria), South Africa. The cells were cultured in sterile minimal essential medium (MEM) supplemented with 0.1% gentamicin and 5% foetal calf serum (FCS) in a 75 cm^2 flask, and incubated at 37°C in 5% CO_2. After a week of incubation, the cells were harvested using 3 ml of trypsin-EDTA, centrifuged for 5 min at 200 x g, counted using a Neubauer haemocytometer, and resuspended in MEM to a concentration of 1 x 10^5 cells per well. The cells (100 µl) were added to all the wells of columns 2-11 of sterile flat bottomed 96 well microtitre plates at a final concentration of 10 000 cells per well, and 200 µl of MEM was added to all the wells of columns 1 and 12. The microtitre plates were incubated at 37°C in 5% CO_2 overnight. The extracts dissolved in 100% DMSO to a concentration of 100 mg/ml were diluted to a concentration ranging from 0.0075 to 1 mg/ml in MEM and added to wells (100 µl) in quadruplicate. Doxorubicin hydrochloride (Pfizer) was used as a positive control, and negative controls (untreated cells, MEM, and DMSO) were also included. The microtitre plates were incubated at 37°C in 5% CO_2 for 48 h. After the 48 h incubation period, the test samples containing MEM were aspirated from the wells, and the cells were washed with 200 µl of phosphate buffered saline (PBS). Fresh MEM (200 µl) and 30 µl of MTT (5 mg/ml stock solution dissolved in PBS) was added to all the wells and the microtitre plates were incubated at 37°C in 5% CO_2 for 4 h. Following 4 h of incubation, 50 µl of DMSO were added to each well, and the plates were shaken gently until the crystals were dissolved. A microplate reader at a wavelength of 570 nm (reference wavelength of 630 nm) was used to measure the amount of MTT reduction. MEM containing wells in columns 1 and 12 were used as blanks. The results were interpreted as percentage of the control wells and LC_{50}

values were noted as the lethal concentration at which 50% of the cells were killed). The following formula was used to calculate percentage cell viability:

$$\text{Percentage cell viability} = \{\frac{\text{Mean absorbance of sample}}{\text{Mean absorbance of control}}\}x100$$

The LC_{50} values were calculated as the concentration of the test sample that resulted in 50% reduction of absorbance compared to untreated cells. From the MIC of the extracts against tested *Mycobacterium* strains and LC_{50} values, the selectivity index values for each extract were calculated using the following formula:

$$SI = \frac{LC50}{MIC}$$

2.4 Genotoxicity

In this study, extracts that showed MIC values less than 1 mg/ml against at least 3 *Mycobacterium* strains were tested for genotoxicity. The *Salmonell*a microsome assay according to Maron and Ames (1983) modified by Mortelmans and Zeiger (2000) was used to determine the genotoxicity of selected extracts. Two *Salmonella typhimurium* tester strains, TA98 and TA100, were used for the assay, which was done without metabolic activation. Briefly, stock bacterial cultures (100 µl) incubated in nutrient broth No.2 at 37°C for 16 h were added (100 µl) to test samples (100 µl) followed by 500 µl of phosphate buffer saline and 2 ml of top agar (2 ml) containing biotin/histidine (0.5 mM). A positive control, 4-nitroquinoline-1-oxide (4-NQO) at a concentration of 2 µg/ml was used, and two negative controls, sterile distilled water and 10% DMSO, were also used. The mixture was vortexed, and poured on minimal agar plates and incubated for 48 h at 37°C. All samples were tested in triplicate. The absence or presence of toxicity was examined by observing the background bacterial growth.

2.5 Phytochemical analysis of selected plant extracts

The phytochemical investigation of selected extracts was done to obtain an overall picture of the number of compounds present in plant extracts by performing thin layer chromatography (TLC) analysis. The extracts were dissolved in their respective solvents of extraction such as acetone, ethanol and water. They were then spotted at the bottom of an aluminium TLC plate coated with silica gel. The TLC plates were developed in eluents made up of hexane:ethyl acetate (8:2 and 7:3), methanol:water (9:1), ethyl acetate: methanol: formic acid (8:2:1) and ethyl acetate: water (10ml:10µl). At the end of each run, the chromatograms were sprayed

with vanillin:sulphuric acid in methanol and dried using a heat gun to visualise the bands. The retention factor (Rf), defined as the distance travelled by the compound divided by the distance travelled by the solvent was determined.

3. Results and Discussion

3.1 Antimycobacterial activity

In vitro antimycobacterial activity of fifty-eight extracts from ten plants; *G. foliosa* (bark), *H. albiflos* (leaves and bulb), *H. trifoliata* (leaves), *H. colchicifolia* (bulb), *P. concolor* (root), *P. acerosum* (leaves and roots), *P. viridiflorum* (bark and leaves), *P. longifolia* (bark and leaves), *P. echinatus* (bark and leaves), and *T. caffrum* (rhizome) against non-pathogenic *Mycobacterium* strains and selected extracts (twenty-nine) against pathogenic strains was determined. The results of the MIC values are presented in Table 2. Although, there are several techniques that are used to determine antimycobacterial activity, an exact cut off value for activity of plant crude extracts has not yet been established. However in previously reported antimycobacterial studies, extracts with MIC values ≤0.1 mg/ml were considered to have significant activity; moderate activity was between 0.1 and 0.63 mg/ml and weak or poor activity with MICs >0.63 mg/ml (Kuete and Efferth, 2010). According to the literature (Molina-Salinas et al., 2006), those extracts with MIC of 125 µg/ml or below are considered active against *Mycobacterium* species. Therefore, analysis of the antimycobacterial activity of crude extracts in the current study was based on the procedures mentioned above. The tested extracts (58) showed a wide range of antimycobacterial activity from significant (eight) to moderate (43) and poor (13) with MIC values ranging from 0.02-2.50 mg/ml against non-pathogenic strains, and the MIC values of twenty-nine extracts tested against pathogenic *Mycobacterium* strains also ranged from 0.02-2.50 mg/ml. The best MIC value=0.02 mg/ml was observed with *P. acerosum* leaves (ethanol; against *M. smegmatis* and *M. bovis* BCG, and water; against *M. bovis* BCG) and roots (ethanol; against *M. smegmatis* and *M. bovis*, and water; against *M. fortuitum* and *M. bovis* BCG) extracts. The extracts of *P. acerosum* roots and leaves showed noteworthy to moderate antimycobacterial activity against all tested *Mycobacterium* strains with MIC values ranging from 0.02 to 0.63 mg/ml. Interesting antimycobacterial activity was observed with the leaf extracts of *H. trifoliata* against *M. bovis* BCG, *M. tuberculosis* H37RV, *M. tuberculosis* (ethanol) and *M. fortuitum* (hot water) at MIC values=0.08 mg/ml. The rest of the extracts of *H. trifoliata* leaves, with the exception of acetone extract, showed moderate antimycobacterial activity against at least 4 non-pathogenic *Mycobacterium* strains, and all the pathogenic strains. Several compounds, namely sarisan, ger

Table 1: Pharmacological studies previously done on the selected traditionally used anti-tuberculosis plant species

Family and botanical name	Voucher specimen number	Pharmacological research done	References
Amaryllidaceae *Haemanthus albiflos* Jacq	PRU122514	Antiviral against Moloney murine leukemia and HIV. Cytotoxicity, phytochemically investigated and yielded homolycorine, albomaculine and O-methyl-lycorenium salt.	Husson et al., 1994, 1995 and 1997, Crouch et al., 2005
Anacardiaceae *Protorhus longifolia* (Bernh.) Engl	PRU122537	Antibacterial, antimycobacterial antiplatelet aggregation, and cytotoxicity activity. Two lanostane triterpenes isolated, tested for antibacterial, antifungal, antiplasmodial, antiplatelet, cytotoxicity, antiinflammatory and antihyperlipidemic properties.	Suleiman et al., 2010; Mosa et al., 2011; Machaba et al., 2014; Kabongo-Kayoka et al., 2016
Apiaceae *Heteromorpha trifoliata* (H.L. Wendl.) Eckl. & Zeyh.	PRU122545	Antibacterial, anthelmintic, antiamoebic, antifungal, genotoxicity activity, and pharmacological effect on smooth muscle evaluated. Antifungal compounds: germacrene-D, sabinene, allylbenzene sarisan and falcarindiol isolated.	Villegas et al., 1988; Mwangi et al., 1994; Katerere and Perry, 2000; McGaw et al., 2000; Adamu et al., 2013
Apocynaceae *Pachycarpus concolor* E. Mey.	B. Madikizela 14	-	
Compositae *Phymaspermum acerosum* (DC.) Källersjö	PRU124356	-	
Celastraceae *Pterocelastrus echinatus* N.E.Br.	PRU122556	-	
Gerrardinaceae *Gerrardina foliosa* Oliv.	B. Madikizela 8	-	
Hypoxidaceae *Hypoxis colchicifolia* Baker	PRU 124355	Anti-inflammatory, antimalarial, anthelmintic, antipyretic, and $GABA_A$-benzodia zepine receptor activity. Norlignan glycosides, geraniol glycoside and β-sitosterol isolated.	Risa et al., 2004; Aremu et al., 2010; Bassey et al., 2014
Pittosporaceae *Pittosporum viridiflorum* Sims	PRU 120025	Antimicrobial, antifungal, anthelmintic, antiamoebic, *in vitro* and *in vivo* (antimalarial and toxicity), antiplasmodial, insecticidal and antiproteus effects. Analysis of phytochemical and antioxidant potential. δ- cadinene, alpha-cadinol, decanal, sabinene, β-elemene and pittoviridoside isolated and tested for antimicrobial activity. Pittoviridoside tested for cytotoxicity.	Ramanandraibe et al., 2001; McGaw et al., 2000; Seo et al., 2002; Clarkson et al., 2004; van Vuuren and Viljoen, 2006; John et al., 2007; Muthaura et al., 2007; Maharaj et al., 2011; Otang et al., 2012; Cock and van Vuuren, 2014
Talinaceae *Talinum caffrum* (Thunb.) Eckl. & Zeyh.	B. Madikizela 1	-	

- =No reported pharmacological studies

Table 2: Antimycobacterial activity of extracts from traditionally used anti-tuberculosis plants in the eastern region of OR Tambo district

Plant species and part used	Extracts	Minimum inhibitory concentration values (mg/ml)							
		Non-pathogenic *Mycobacterium* strains					Pathogenic *Mycobacterium* strains		
		M. sm	***M. fo***	***M. au***	***M. bo* BCG**	***M. go***	***M. bo***	***M. tb* Rv**	***M. tb***
Gerrardina foliosa (bark)	Acetone	0.63	0.63	1.25	2.50	1.25	-	-	-
	Ethanol	1.25	2.50	0.63	2.50	1.25	-	-	-
	Water	1.25	2.50	0.63	1.25	2.50	-	-	-
	Hot water	1.25	0.31	0.63	0.63	2.50	0.63	0.31	0.31
Haemanthus albiflos (leaf)	Acetone	0.63	0.63	2.50	2.50	2.50	-	-	-
	Ethanol	2.50	2.50	2.50	2.50	2.50	-	-	-
	Water	2.50	2.50	2.50	2.50	2.50	-	-	-
Haemanthus albiflos (bulb)	Acetone	1.25	2.50	2.50	0.63	0.63	-	-	-
	Ethanol	2.50	1.25	2.50	2.50	2.50	-	-	-
	Water	1.25	1.25	2.50	2.50	2.50	-	-	-
Heteromorpha trifoliata (leaf)	Acetone	1.25	1.25	1.25	0.31	1.25	2.50	1.25	5.00
	Ethanol	0.63	0.31	0.63	**0.08**	**0.08**	0.16	**0.08**	**0.08**
	Water	0.63	0.63	0.63	0.63	0.63	0.31	0.16	0.16
	Hot water	0.63	**0.08**	0.63	2.50	0.63	0.63	0.16	0.31
Hypoxis colchicifolia (bulb)	Acetone	0.63	0.63	0.63	0.63	0.63	0.31	0.31	0.63
	Ethanol	1.25	2.50	0.63	0.63	0.63	0.63	0.31	0.31
	Water	0.63	0.63	0.63	0.31	0.31	0.63	0.31	0.16
	Hot water	0.31	**0.08**	0.63	0.31	0.31	0.16	0.31	0.31
Pachycarpus concolor (root)	Acetone	1.25	1.25	2.50	0.31	1.25	-	-	-
	Ethanol	1.25	2.50	2.50	2.50	2.50	-	-	-
	Water	1.25	2.50	1.25	2.50	2.50	-	-	-
	Hot water	2.50	2.50	1.25	0.31	1.25	-	-	-
Phymaspermum acerosum (leaf)	Acetone	0.31	0.31	0.31	0.31	0.16	0.63	0.31	0.63
	Ethanol	**0.02**	0.16	0.16	**0.02**	0.16	**0.02**	0.16	0.16
	Water	0.16	**0.08**	**0.08**	**0.02**	0.16	**0.04**	**0.08**	**0.08**
Phymaspermum acerosum (root)	Acetone	0.16	0.16	**0.08**	0.16	0.31	0.31	0.31	0.16
	Ethanol	**0.02**	0.31	**0.08**	**0.02**	0.63	0.16	0.31	0.31
	Water	**0.08**	**0.02**	**0.08**	**0.02**	0.63	**0.04**	**0.04**	**0.04**

Pittosporum viridiflorum (leaf)	Acetone	0.63	2.50	1.25	0.31	0.31	0.16	0.31	0.31
	Ethanol	2.50	0.63	1.25	0.31	0.31	0.63	1.25	2.50
	Water	1.25	0.63	2.50	0.31	2.5	-	-	-
	Hot water	2.50	2.50	2.50	0.31	2.5	-	-	-
Pittosporum viridiflorum (bark)	Acetone	0.63	0.63	2.50	0.63	1.25	0.63	2.50	2.50
	Ethanol	0.63	0.31	2.50	0.63	1.25	0.63	1.25	1.25
	Water	0.63	0.63	0.63	0.63	1.25	0.63	1.25	1.25
	Hot water	2.50	2.50	2.50	0.63	1.25	-	-	-
Protorhus longifolia (bark)	Acetone	2.50	2.50	2.50	1.25	1.25	-	-	-
	Ethanol	2.50	2.50	2.50	1.25	2.50	-	-	-
	Water	2.50	2.50	2.50	0.31	2.50	-	-	-
	Hot water	2.50	0.16	0.63	0.63	1.25	-	-	-
Protorhus longifolia (leaf)	Acetone	**0.08**	0.16	0.16	**0.08**	0.16	0.16	0.31	0.16
	Ethanol	0.31	0.31	0.31	0.16	1.25	0.63	1.25	1.25
	Water	0.63	0.63	1.25	2.50	1.25	-	-	-
	Hot water	0.63	0.63	0.31	2.50	1.25	0.31	0.16	0.31
Pterocelastrus echinatus (bark)	Acetone	0.31	0.31	0.63	0.31	0.63	0.16	0.16	0.31
	Ethanol	0.31	0.31	0.63	0.63	0.63	0.31	0.31	0.63
	Water	1.25	2.50	1.25	0.63	1.25	-	-	-
	Hot water	0.63	0.63	0.31	0.31	0.63	0.31	0.16	0.16
Pterocelastrus echinatus (leaf)	Acetone	0.63	0.63	1.25	0.63	0.63	0.63	0.31	0.31
	Ethanol	0.31	0.31	0.63	0.63	0.63	0.63	0.16	0.16
	Water	0.63	1.25	1.25	0.63	0.63	0.31	1.25	0.63
	Hot water	1.25	2.50	2.50	1.25	1.25	0.63	1.25	0.31
Talinum caffrum (rhizome)	Acetone	1.25	1.25	2.50	2.50	2.50	-	-	-
	Ethanol	2.50	2.50	1.25	2.50	2.50	-	-	-
	Water	1.25	1.25	2.50	2.50	2.50	-	-	-
	Hot water	2.50	1.25	2.50	2.50	2.50	-	-	-
Isoniazid		> 0.1	> 0.1	> 0.1	> 0.1	> 0.1		0.03	
Rifampicin		0.03	0.03	0.01	0.03	0.01		0.08	
Streptomycin		> 0.1	> 0.1	> 0.1	> 0.1	> 0.1			

M. sm= Mycobacterium smegmatis, *M. fo= Mycobacterium fortuitum*, *M. au= Mycobacterium aurum*, *M. bo* BCG= *Mycobacterium bovis* BCG, *M. go= Mycobacterium gordonae*, *M. tb* Rv= *Mycobacterium tuberculosis* H37RV, *M. tb= Mycobacterium tuberculosis*, *M. bo= Mycobacterium bovis*, -=not tested. Values in bold represent good activity (MIC < 0.1 mg/ml)

macrene-D, sabinene, allylbenzene and falcarindiol have reportedly been isolated from *H. trifoliata* leaves and they could be responsible for the observed antimycobacterial activity (Villegas et al., 1988; Recio et al., 1995). Falcarindiol previously isolated from the whole herb of *Anethum graveolens* showed noteworthy antimycobacterial activity against *M. fortuitum, M. aurum, M. smegmatis, M. abscessus* and *M. phlei* with MIC values ranging from 2-4 µg/ml, thus making the compound a potential target for the development of anti-TB drugs (Stavri and Gibbons, 2005). *H. colchicifolia* (bulb) hot water extract showed noteworthy antimycobacterial activity against *M. fortuitum* with MIC value=0.08mg/ml, and all the extracts showed moderate activity towards at least 6 *Mycobacterium* strains. A phenolic glycoside (3-hydroxy-4-O-β-D-glucopyranosylbenzaldehyde), geraniol glycoside and β-sitosterol were reported to have been isolated from the bulbs of *H. colchicifolia* and they could be responsible for the antimycobacterial activity observed from this plant, but confirmation of that is still pending (Bassey et al., 2014). For *P. longifolia*, the acetone leaf extracts showed interesting activity against *M. smegmatis* and *M. bovis* BCG with MIC=0.08 mg/ml, and moderate antimycobacterial activity was observed with the ethanol, and hot water extracts of the same plant against at least 4 *Mycobacterium* strains. Just like in this study, the leaf acetone extract of *P. longifolia* showed significant antimycobacterial activity against *M. smegmatis* in previous work (Kabongo-Kayoka et al., 2016). The bark is the reported plant part of *P. longifolia* for traditional use against TB. Although, most extracts from this plant were inactive, it was interesting to observe antimycobacterial activity of the hot water extract against at least 6 strains, although moderate ranging from 0.16-0.63 mg/ml.

Both the bark (acetone, ethanol and hot water) and leaf (acetone, ethanol and water) extracts of *P. echinatus* showed moderate antimycobacterial activity against at least five *Mycobacterium* strains. Homolycorine, albomaculine, albimanthine, and O-methyl-lycorenium salt reportedly isolated from *H. albiflos* (Baudoin et al., 1994; Crouch et al., 2005) could be responsible for moderate antimycobacterial activity shown by the leaf acetone extracts of this plant (against *M. smegmatis* and *M. fortuitum*) and bulbs (against *M. bovis* BCG and *M. gordonae*). The bark and leaf extracts of *P. viridiflorum* demonstrated moderate activity towards at least one strain of *Mycobacterium*. Pittoviridoside (Seo et al., 2002), sesquiterpene glycoside and its derivatives (Ramandraibe et al., 2001), pentacyclic triterpenoids (Nyabayo et al., 2015), and pentacyclic triterterpenoiol estersaponin (Nyongbela et al., 2013) reportedly isolated from the leaves of this plant could be responsible for the observed activity. For *P. concolor*, only the acetone extract showed moderate antimycobacterial activity towards at least one

strain (*M. bovis* BCG, MIC=0.31 mg/ml), while the rest of the extracts showed poor activity. There are different compounds within an extract that may act in synergy, but once isolated, they might demonstrate more activity than the crude extract, therefore, moderate antimycobacterial activity of crude extracts is worthy of further investigation.

Interesting antimycobacterial activity observed with ethanol and water extracts of *P. acerosum*, *H. trifoliata* and *H. colchicifolia* was encouraging as these are the solvents used for extraction in African traditional medicine, providing some credence for the traditional use of these plants against TB. In spite of the report of *T. caffrum* for traditional anti-TB use, all the extracts from this plant showed poor antimycobacterial activity, and this could be that anti-TB effects of this plant are mediated through immunomodulation or immunostimulation rather than direct mycobacterial inhibition, or that the potentially active constituents from this plant are pH-dependent or need direct *in vivo* specific enzymes activation (Ríos and Recio, 2005). Therefore, the observed poor antimycobacterial activity of *T. caffrum* in this study could not preclude its potential anti-TB effect. The pharmacological activities of the plants tested for antimycobacterial potential in this study are displayed in Table 1, and to the best of our knowledge, this is the first report of the antimycobacterial activity of almost all the plants tested, with the exception of *P. longifolia* leaf acetone extract. Additionally, no compounds have been isolated from *P. acerosum*, *P. echinatus*, *G. foliosa*, and *P. concolor*, therefore, investigation of the constituents from these plants that are responsible for their antimycobacterial activity is highlighted as a worthy future endeavour.

Destructive harvesting of underground plant parts, slow-growth of bulbous and tuberous plants, and tree ring-barking are the major problems in traditional medicine, as some of the plants may not be available for use when needed, due to rarity or extinction. One of the possible solutions proposed by plant conservationists to fight plant extinction is alternative plant part use, particularly encouraging traditional healers to use leaves and twigs instead of bark, roots or bulbs. In this study we prepared extracts from alternative plant parts for some of the plants that were tested to determine if the same activity could be observed from a different plant part, particularly the leaf. For *P. acerosum*, the leaf extracts showed inhibition of *Mycobacterium* strains against all the strains tested comparable to root which is the plant part reported for traditional anti-TB use, and importantly the activity of leaf water extract should be noted, as it could be an alternative plant part proposed to traditional healers for use against TB. Comparing the leaf and bark extracts for *P. viridiflorum*, *P. longifolia* and *P. echinatus*,

the leaves demonstrated antimycobacterial activity although the bark was reported for traditional use against TB. For *Haemanthus albiflos* leaf and bulb extracts, both plant parts showed comparable results in terms of antimycobacterial activity, with the acetone extracts showing MIC values=0.63 mg/ml against two *Mycobacterium* strains although the bulb was reported to be the plant part used in traditional anti-TB therapy in the eastern region of OR Tambo district. Therefore, these results support plant part substitution, and more in depth studies should investigate whether the leaf, as an alternative part from these plants, has the same secondary metabolites as the bark/roots/stem/bulb.

The findings for predictor of activity against pathogenic *Mycobacterium* strains amongst the non-pathogenic *Mycobacterium* strains used in this study are presented in Table 3 as correlation coefficient (r) values. Positive correlations were observed between the activity of non-pathogenic and pathogenic *Mycobacterium* strains with r values ranging from 0.02-0.73, whereas negative correlation was observed only with *M. bovis* BCG on *M. tuberculosis* (r value=-0.05). *M. gordonae*, *M. aurum*, *M. smegmatis* showed good correlation of activity with *M. tuberculosis* H37Rv, *M. tuberculosis* and *M. bovis*, however, the highest correlation value was observed on *M. aurum* (r value=0.73), suggesting that this strain was a better predictor of activity for pathogenic *M. tuberculosis* H37Rv in this study among the non-pathogenic strains. *M. smegmatis* was observed to be a better predictor of activity of *M. bovi*s and *M. tuberculosis* with r values=0.54 and 0.49, respectively. However, in contrast to our finding *M. bovis* BCG was reported as a better predictor of activity with *M. tuberculosis* when compared to *M. smegmatis* (Altaf et al., 2010). In agreement with our study, *M.* a*urum* was reported as a better predictor of anti-*M. tuberculosis* activity by Aro et al. (2015). In this study, the highest r value=0.83 was observed with pathothogenic *M. bovis* and *M. tuberculosis*. More in depth studies determining which one of the non-pathogenic *Mycobacterium* strains is a best predictor of activity towards pathogenic *Mycobacterium* strains are requested

3.2 Thin layer chromatography (TLC) analysis, a quick chemical fingerprinting of the plant extracts

Twelve crude extracts with noteworthy antimycobacterial activity against pathogenic strains were selected for the study so as to obtain their fingerprints, and the results are presented in Figures 1a-1d. Analysis of *P. acerosum* leaf and root (acetone and ethanol) extracts in hexane and ethyl acetate (8:2) showed the presence of at least 3 nonpolar compounds, with two that

Table 3: Pearson's correlation coefficient (r) between minimum inhibitory concentration values of eight tested *Mycobacterium* strains

	M.s	*M. fo*	*M. au*	*M. bo* BCG	*M. go*	*M. bov*	*M. tb* Rv	*M. tb*
M.s	1							
M. fo	0.67	1						
M. au	0.66	0.55	1					
M. bo BCG	0.33	0.32	0.43	1				
M. go	0.62	0.50	0.67	0.63	1			
M. bo	**0.54**	**0.25**	**0.35**	**0.12**	**0.41**	1		
M. tb Rv	**0.38**	**0.25**	**0.73**	**0.02**	**0.42**	0.44	1	
M. tb	**0.49**	**0.14**	**0.43**	**-0.05**	**0.31**	0.83	0.69	1

M. sm= Mycobacterium smegmatis, M. fo= Mycobacterium fortuitum, M. au= Mycobacterium aurum, M. bo BCG= *Mycobacterium bovis* BCG, *M. go= Mycobacterium gordonae*, *M. tb* Rv= *Mycobacterium tuberculosis* H37RV, *M. tb= Mycobacterium tuberculosis, M. bo= Mycobacterium bovis*,

seemed to be similar based on the Rf values observed (Figure 1a). The water extracts of *P. acerosum* (leaf and root) showed 3 highly polar compounds in ethyl acetate and water (10 ml:30 µl) with one common in both samples with the Rf value=0.55 (Figure1d). As seen in figure 1b, *P. longifolia* leaf acetone extract showed 5 non-polar compounds, with one major which had an Rf value=0.88, and according to literature, non-polar compounds have been isolated from this plant part (Ntuli, 2005). Four non-polar compounds were observed on *P. echinatu*s bark hot water extracts (Figure 1b) and highly polar compounds were observed on the leaf ethanol extracts of the same plant (Figure 1c). Ethanol and water leaf extracts of *H. trifoliata* showed at least 2 polar compounds (Figure 1c), and two polar compounds were reportedly isolated from the leaf methanol extract of the same plant (Recio et al, 1995).

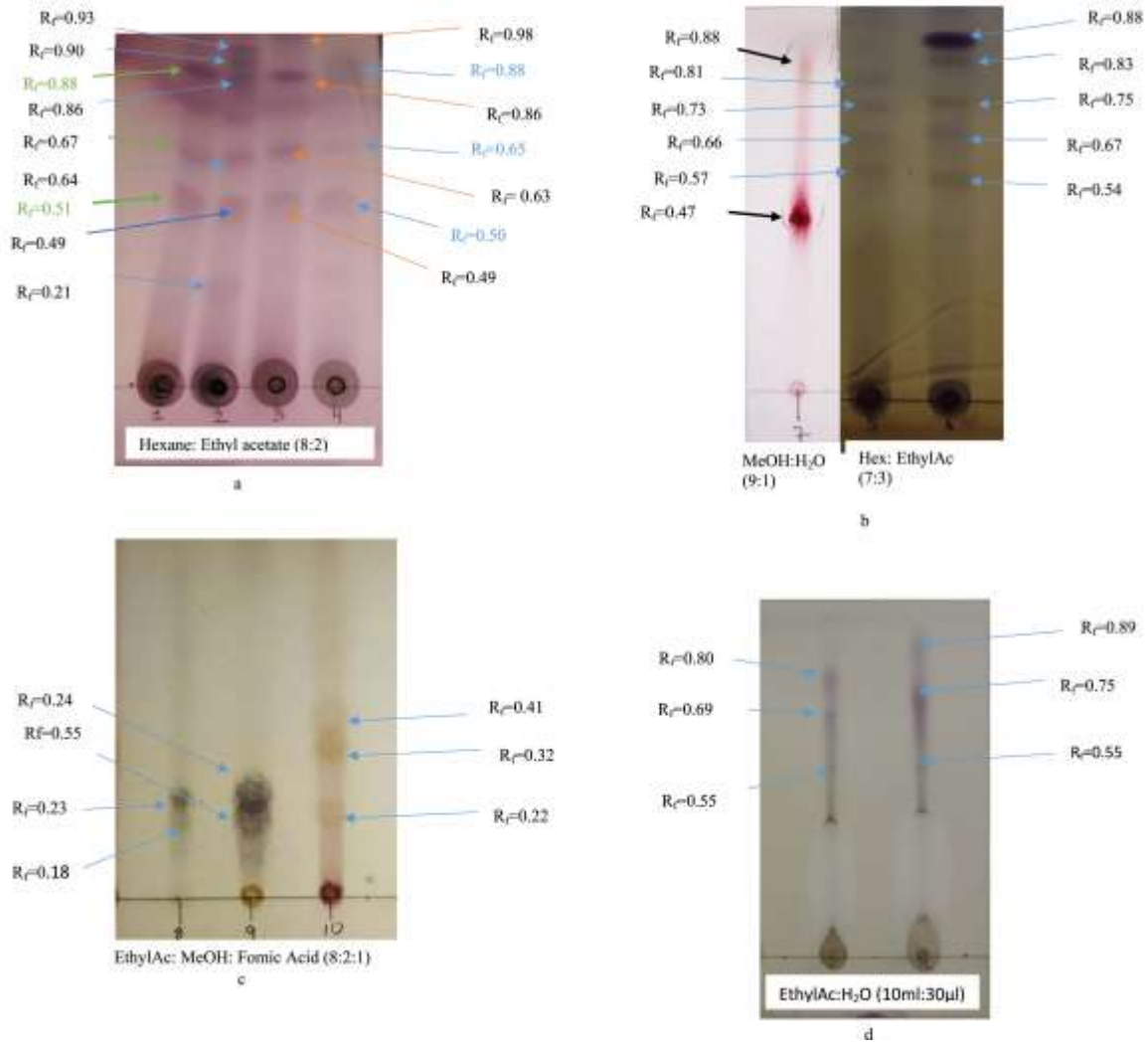


Fig. 1. a: TLC profiling of *P. acerosum* leaf (1 = acetone, 3 = ethanol) and root (2 = acetone, 4 =ethanol) extracts. b: TLC profiling of *P. echinatus* bark (7 = hot water) and leaf (5 = acetone), as well as *P. longifolia* leaf (6 = acetone) extracts. c: TLC profiling of *H. trifoliata* leaf (8 = ethanol, 9 = water) and *P. echinatus* leaf (10 = ethanol) extracts. d: TLC profiling of *P. acerosum* leaf (11 = water) and root (12 = water) extracts.

3.3 Cytotoxicity

Although several studies have led to the validation of some plant derived traditional remedies, research has demonstrated that medicinal plants are potentially toxic in both *in vitro* and *in vivo* assays, thus they should be used cautiously (Santin et al., 2011). *In vitro* cytotoxicity studies should be done at the early stages of the drug development process as a preliminary indicator of potential toxicity. In this study, plants showing MIC values less than 1 mg/ml against at least three *Mycobacterium* strains were investigated further for cytotoxicity against Vero monkey and bovine dermis cells. The results for cytotoxicity of selected crude extracts represented as LC_{50} values, and selectivity index values, which show the relationship between antimycobacterial activity and toxicity, are shown in Table 4. Crude extracts with LC_{50} values >0.1 mg/ml are considered non-cytotoxic (Kuete and Efferth, 2010).

Cytotoxicity varied in this study, and some selectivity index values were highly promising. The LC_{50} values of twenty-nine extracts tested ranged from 0.001-5.59 mg/ml, and the selectivity index values ranged from 0.003-79.50. According to toxicity standards mentioned above, the tested extracts of *G. foliosa*, *H. colchicifolia*, *H. trifoliata*, *P. acerosum*, *P. viridiflorum* (bark), *P. echinatus* (leaf), and *P. longifolia* (bark) were non-cytotoxic against both Vero and bovine dermis cells with LC_{50} values ranging from 0.12 to >1 mg/ml, and the highest selectivity index value of 79.50 was demonstrated by *P. acerosum* root water extract. A high selectivity index value marks a large safety margin between the concentration of the test sample that is able to inhibit the growth of *Mycobacterium* strains and the concentration that is toxic to BD and Vero cells in this case. Therefore, selection of an extract with a high selectivity index value for further analysis increases the chances of producing a useful herbal medicine.

The bark acetone and hexane extracts of *P. viridiflorum* were previously reported to be non-toxic when tested against *Artemia salina* larval and hatching stages (Otang et al., 2013), to some extent supporting the safety of this plant part. Selectivity index values greater than 1 for *P. acerosum* (ethanol and water), *H. colchicifolia* (cold and hot water), *H. trifoliata* (ethanol, cold water and hot water) and *P. longifolia* (leaf acetone) extracts indicated a wide safety margin. This provides a measure of support for the traditional use of these plants against TB, and creates an urgent need for identification of safe antimycobacterial compounds from them. Lack of toxicity with extracts of *H. trifoliata*, *P. viridiflorum* (leaves) and *H. colchicifolia*

Table 4: LC_{50} and selectivity index values of selected extracts against Vero monkey kidney and bovine dermis cells

Plant species	Ext	LC_{50} values against Vero	Selectivity index values Vero								LC_{50} values against BD	Selectivity index values BD							
			M. sm	*M. fo*	*M. au*	*M. bo BCG*	*M. go*	*M. bo*	*M. tb* Rv	*M. tb*		*M. sm*	*M. fo*	*M. au*	*M. bo BCG*	*M. go*	*M. bo*	*M. tb* Rv	*M. tb*
G. foliosa(Ba)	HW	0.69±1.02	0.552	2.23	1.10	1.10	2.23	1.10	2.23	2.23	0.28±.0.20	0.22	0.90	0.44	0.44	0.22	0.44	0.90	0.90
H. colchicifolia(Bu)	A	0.98±0.24	1.56	1.56	1.56	1.56	1.56	3.16	3.16	1.56	0.14±0.07	0.22	0.22	0.22	0.22	0.22	0.45	0.45	0.22
	E	0.89±0.45	0.71	0.36	1.41	1.41	1.41	1.41	2.87	2.87	0.19±1.04	0.15	0.076	0.30	0.30	0.30	0.30	0.61	0.61
	W	2.48±0.02	3.94	3.94	3.94	3.94	3.94	3.94	8.00	**15.50**	1.36±0.54	0.63	2.16	2.16	4.39	4.39	2.16	4.39	8.50
	HW	0.39±0.44	1.25	2.50	0.62	1.25	1.25	2.43	1.26	1.26	0.36±0.27	1.16	4.50	0.57	1.16	1.16	2.25	1.16	1.16
H. trifoliata(L)	E	5.59±0.17	8.94	**17.88**	8.94	**35.83**	**35.83**	**34.94**	**69.88**	**69.88**	1.43±0.98	2.27	4.61	2.27	**17.88**	**17.88**	8.94	**17.88**	**17.88**
	W	3.68±1.06	5.84	5.84	5.84	5.84	5.84	**11.87**	**23.00**	**23.00**	0.89±0.37	1.41	1.41	1.41	1.41	1.41	2.87	5.56	5.56
	Hw	3.22±1.28	5.10	**20.58**	5.10	1.28	5.10	5.11	**20.13**	**10.39**	1.33±0.54	2.11	**16.63**	2.11	0.53	2.11	2.11	8.31	4.29
P. acerosum(L)	A	0.59±0.82	0.94	0.94	0.94	0.94	3.68	0.94	1.90	0.94	0.25±0.48	0.81	0.81	0.81	0.81	1.56	0.40	0.81	0.40
	E	0.28±0.78	**14.00**	1.75	1.75	**14.00**	1.75	**14.00**	1.75	1.75	0.46±1.06	**23.00**	2.88	2.88	**23.00**	2.88	**23.00**	2.88	2.88
	W	0.99±0.26	**16.19**	**12.38**	**12.38**	**49.50**	6.19	**24.75**	**12.38**	**12.38**	0.87±1.08	5.44	**10.88**	**10.88**	**43.50**	5.44	**21.75**	**10.88**	**10.88**
P. acerosum(R)	A	0.38±0.45	2.38	2.38	4.75	2.38	1.23	1.23	1.23	2.38	0.12±1.18	0.75	0.75	1.50	0.75	0.39	0.39	0.39	0.75
	E	0.41±0.76	**20.50**	1.32	5.13	0.65	1.32	2.56	1.32	1.32	0.24±0.37	**12.00**	0.77	3.00	12.00	0.38	1.50	0.77	0.77
	W	0.59±0.86	6.88	**27.50**	6.88	27.50	0.87	**14.75**	**14.75**	**14.75**	1.59±0.50	**19.88**	**79.50**	**19.88**	**79.50**	2.52	**39.75**	**39.75**	**39.75**
P. viridiflorum (Ba)	A	0.47±0.87	0.75	0.75	0.19	0.75	0.38	0.74	0.19	0.19	0.37±0.67	0.58	0.58	0.15	0.58	0.30	0.59	0.15	0.15
	E	0.91±1.28	0.63	0.31	2.50	0.63	1.25	1.44	0.73	0.73	1.41±0.08	2.24	4.55	2.50	2.24	1.13	2.24	1.13	1.13
	W	1.44±1.80	2.29	2.29	2.29	2.29	1.15	2.29	1.15	1.15	0.20±1.02	0.32	0.32	0.32	0.32	0.16	0.32	0.16	0.16
P. viridiflorum(L)	A	0.96±0.82	1.52	0.38	0.77	3.10	3.10	6.00	3.10	3.10	0.04±1.22	0.06	0.02	0.03	0.13	0.13	0.25	0.13	0.13
	E	0.95±1.37	0.38	1.51	0.38	3.06	3.06	1.51	0.76	0.38	0.08±0.37	0.03	0.13	0.06	0.26	0.26	0.13	0.06	0.03
P. echinatus(L)	A	0.18±0.58	0.29	0.29	0.14	0.29	0.29	0.29	0.58	0.58	1.12±1.48	1.78	1.78	0.90	1.78	1.78	1.78	3.61	3.61
	E	0.24±0.11	0.77	0.77	0.38	0.38	0.38	0.38	1.50	1.50	0.14±0.37	0.45	0.45	0.22	0.22	0.22	0.22	0.88	0.88
	W	0.46±0.18	0.73	0.37	0.37	0.73	0.73	1.48	0.37	0.73	0.30±1.08	0.48	0.24	0.24	0.48	0.48	0.97	0.24	0.48
P. echinatus(Ba)	A	0.001±0.24	0.003	0.003	0.002	0.003	0.002	0.01	0.01	0.00	0.95±0.26	1.51	1.51	0.76	1.51	1.51	5.93	5.93	3.06
	E	0.34±0.40	1.10	1.10	0.54	0.54	0.54	1.10	1.10	0.54	0.06±0.18	0.19	0.19	0.10	0.10	0.10	0.19	0.19	0.10
	Hw	0.50±0.11	0.79	0.79	1.61	0.79	0.79	0.79	0.40	1.61	0.01±1.02	0.02	0.02	0.03	0.03	0.02	0.02	0.01	0.03
P. longifolia(L)	A	0.87±0.37	**10.88**	5.44	5.44	**10.88**	5.44	5.43	2.81	5.43	0.34±0.32	4.25	2.13	2.13	4.25	2.13	2.13	1.10	2.13
	E	0.04±1.08	0.13	0.13	0.13	0.25	0.03	0.06	0.03	0.03	1.18±0.13	3.80	3.80	3.80	7.38	0.94	1.87	0.94	0.94
	HW	0.02±1.02	0.03	0.03	0.06	0.06	0.03	0.06	0.13	0.06	0.008±0.18	0.01	0.01	0.03	0.03	0.01	0.03	0.05	0.03
P. longifolia(Ba)	HW	0.23± 0.40	0.09	1.44	0.37	0.37	0.18	0.74	1.44	1.44	0.86±1.08	0.34	5.37	1.37	1.37	0.69	2.77	5.38	5.38
Doxorubicin		0.007±0.50									0.23±0.07								

Ba= bark, Bu=bulb, L= leaf, R= root, BD= Bovine dermis cells, Vero=Vero monkey kidney cells LC_{50}= the lethal concentration at which 50% at which cells are killed, *M. sm= Mycobacterium smegmatis*, *M. fo= Mycobacterium fortuitum*, *M. au= Mycobacterium aurum*, *M. bo* BCG= *Mycobacterium bovis* BCG, *M. go= Mycobacterium gordonae*, *M. tb* Rv= *Mycobacterium tuberculosis* H37RV, *M. tb= Mycobacterium tuberculosis*, *M. bo= Mycobacterium bovis G. foliosa= Gerrardina foliosa*, *H. trifoliata= Heteromorpha trifoliata*, *H. colchicifolia= Hypoxis colchicifolia*, *P. acerosum= Phymaspermum acerosum*, *P. viridiflorum= Pittosporum viridiflorum*, *P. longifolia= Protorhus longifolia*, *P. echinatus= Pterocelastrus echinatus.* Values in bold represent excellent selectivity index values (> 10).

towards Vero cells confirmed previous cytotoxicity findings by Adamu et al. (2013) and Verschaeve et al. (2013). Although non-cytotoxic, the selectivity index values of some of the extracts were lower than 1; *G. foliosa* (bark hot water), *P. viridiflorum* (bark acetone and water), *H. colchicifolia* (bulb acetone and ethanol), and *P. echinatus* (leaf ethanol and water) towards either both cells tested or one of them, suggesting that these extracts might be unsafe for use. The leaf acetone extract of *P. longifolia* was reported previously by Kabongo-Kayoka et al. (2016) to have no toxicity at the highest concentration tested towards Vero, C3A and RAW 264.7 cells with LC_{50} values ranging from 0.62- >1 mg/ml supporting the current safety findings of this plant extract. Furthermore, the chloroform leaf extract of *P. longifolia* was reported to be non-cytotoxic against equine erythrocytes, and Vero cells in previous research by Mosa et al. (2011). For *P. longifolia,* the bark ethanol extract showed selective toxicity towards Vero cells (LC_{50}=0.04), whereas the water extract was slightly toxic against both Vero and bovine dermis cells with LC_{50} values of 0.06 and 0.01 mg/ml, respectively. These plant extracts should be used with caution in traditional anti-TB therapy. On a positive note, two triterpene lactones isolated from the bark of *P. longifolia* tested against human embryonic kidney and human hepatocellular carcinoma were reported to be insignificantly cytotoxic (Mosa et al. 2011), suggesting the safety of some of the constituents of this plant.

P. echinatus bark acetone (against Vero cells) and *P. longifolia* hot water (against bovine dermis cells) extracts were highly toxic with LC_{50} values of 0.001 and 0.008 mg/ml respectively, and these two extracts displayed more toxicity towards the cells than antimycobacterial activity, as their selectivity index values were less than 1, ranging from 0.002-0.03. The bark ethanol and hot water extracts of *P. echinatus* also showed differential toxicity towards bovine dermis cells with LC_{50} values of 0.06 and 0.01 mg/ml, respectively. Although the leaf acetone extract of *P. viridiflorum* was previously reported by Adamu et al. (2013) to have no cytotoxicity against Vero cells, in the current study the same extract and the ethanol extract was toxic to bovine dermis cells with LC_{50} values of 0.04 and 0.08 mg/ml, respectively. Additionally, the acetone, hexane, methanol, and water extracts from *P. viridiflorum* leaves showed cytotoxicity against the Chang liver cell line in previous studies by Seo et al. (2002), Muthaura et al. (2007) and Otang et al. (2014). The cytotoxicity results observed on bark *P. echinatus* (acetone), *P. longifolia* (hot water and ethanol) extracts suggests that they might be unsafe for traditional use against TB, however more in depth cytotoxicity research on these

extracts which should include determining their *in vivo* cytotoxicity is required as they might respond differently.

3.4 Genotoxicity

Evaluation of the mutagenic effects of medicinal plants is highly recommended for detecting potential genotoxins, and the *Salmonella* based Ames test is used primarily to determine safety of test samples. The *Salmonella typhimurium* tester strains TA98 and TA100 are recommended for general genotoxicity testing as they highlight frame-shift and base-pair substitution gene mutations, respectively. In total, twenty-nine extracts were selected for the genotoxicity study, and the results are shown in Table 5. To say that an extract is genotoxic, the number of revertant colonies should be more than twice that of the negative control and a clear dose-dependent response should be observed for the various concentrations tested (Maron and Ames, 1983). Out of the extracts tested, almost all of them were negative in the Ames test with the exception of *P. viridiflorum* bark water and *H. trifoliata* leaf ethanol extracts that showed clear mutagenicity with numbers of revertant colonies greater than twice that of the negative controls for the 5 mg/ml, the highest concentration tested, however decreasing with decreasing concentrations of extracts. The extracts of *P. acerosum* (leaf acetone), and *P. viridiflorum* (bark acetone and ethanol), although not toxic against *S. typhimurium* TA98, showed a dose-dependent response against *S. typhimurium* TA100. According to our knowledge, although *P. viridiflorum* has been tested for toxicity both in *in vitro* and *in vivo* studies, this is the first report of the genotoxicity testing of this plant (Madikizela and McGaw, 2017). The observed lack of genotoxicity of water extracts of *H. colchicifolia* and *H. trifoliata* (against TA98) confirmed previous findings (Elgorashi et al., 2003; Verschaeve and Van Staden, 2008; Verschaeve et al., 2013) when tested against one strain. However, *H. trifoliata* ethanol extract showed genotoxicity against TA100 in the current study. Therefore, the use of *P. viridiflorum* should be treated with caution and rigorous toxicological studies are necessary. For more in depth genotoxicity investigations on these plants, subsequent tests should be conducted, and they should include metabolic activation as well as other strains such as TA97, TA1535, TA102 and TA104.

4. Conclusions

This study reported for the first time the antimycobacterial potential of *Gerrardina foliosa*, *Haemanthus albiflos*, *Heteromorpha trifoliata*, *Hypoxis colchicifolia*, *Pachycarpus concolor*, *Phymaspermum acerosum*, *Pittosporum viridiflorum*, *Protorhus longifolia* (bark), *Pterocelas-*

Table 5: Genotoxicity of selected extracts against *Salmonella typhimurium* strains (TA98 and TA100) presented as mean± standard error

Plant species	Extract	*Salmonella typhimurium* strain					
		TA 98			TA 100		
		Concentrations (mg/ml)					
		5 mg/ml	0.5 mg/ml	0.05 mg/ml	5 mg/ml	0.5 mg/ml	0.05 mg/ml
		Number of revertant colonies per concentration					
G. foliosa (bark)	Hot water	35.00±0.53	31.00±1.53	37.00±1.67	228.50±1.50	211.33±1.09	227.00±1.000
H. colchicifolia (bulb)	Acetone	29.67±0.33	30.33±0.33	32.33±0.33	231.00±1.00	233.00±0.58	243.00±0.58
	Ethanol	33.33±1.13	29.33±0.67	27.67±0.67	319.00±1.00	318.33±1.90	251.00±0.58
	Water	35.33±0.63	31.33±0.66	29.33±0.33	243.00±1.53	246.00±0.58	246.67±0.33
	Hot water	33.33±0.33	29.33±0.67	27.67±0.67	335.00±1.52	306.33±1.49	310.67±0.88
H. trifoliata (leaf)	Ethanol	32.67±0.88	36.33±0.67	30.67±0.33	458.67 ±1.00	202.33±0.33	260.67±0.67
	Water	34.67±0.43	33.33±0.03	35.33±0.18	257.67±0.33	256.00±0.58	247.33±1.33
	Hot water	30.33±0.64	31.33±1.63	39.33±0.33	293.00±0.33	296.00±0.58	296.67±0.33
P. acerosum (leaf)	Acetone	32.00±1.11	39.33±1.33	30.33±0.88	255.67±1.45	214.33±2.66	209.33±3.33
	Ethanol	38.33±0.67	37.000±1.00	35.67±0.88	258.33±2.67	231.67±3.28	224.00±3.00
	Water	38.67±0.33	28.00±1.15	32.00±0.58	232.33±1.33	206.00±1.00	199.67±2.67
P. acerosum (root)	Acetone	45.00±1.67	31.00±1.53	37.00±2.08	269.67±1.67	245.00±2.00	223.00±2.52
	Ethanol	41.67±1.88	42.33±1.33	40.21±1.36	259.67±0.66	236.67±1.33	219.67±0.33
	Root water	30.00±0.50	32.00±0.00	31.67±0.33	214.67±0.67	267.33±1.33	190.33±2.33
P. viridiflorum (bark)	Acetone	36.00±1.00	30.67±0.88	36.00±1.73	323.67±1.48	295.00±0.00	236.00±0.00
	Ethanol	36.33±0.88	31.33±1.33	35.00±0.00	379.67±1.67	309.33±1.33	320.33±1.12
	Water	64.33±0.33	34.67±0.33	23.00±0.00	817.00±000	390.33±0.34	130.33±1.45
P. viridiflorum (leaf)	Acetone	33.33±0.33	39.00±1.00	32.33±0.33	272.33±0.67	236.00±1.00	326.33±1.83
	Ethanol	35.33±0.63	37.00±0.66	34.33±0.30	260.33±0.42	226.00±0.30	220.03±1.83
P. echinatus (bark)	Acetone	36.67±0.33	36.00±0.00	30.00±0.00	229.33±0.88	243.00±0.00	249.00±0.58
	Ethanol	39.67±0.33	35.33±0.33	37.33±0.33	257.00±0.58	235.67±0.88	233.33±0.33
	Hot water	32.17±1.03	33.03±1.04	27.23±1.67	200.08±0.58	215.08±0.88	210.23±0.33
P. echinatus (leaf)	Acetone	35.33±0.32	32.67±0.30	35.33±0.67	284.33±0.33	207.33±0.18	244.00±0.06
	Ethanol	38.33±0.32	34.67±0.30	36.33±0.67	284.33±0.33	207.33±0.18	244.00±0.06
	Water	35.00±1.33	31.00±0.53	27.00±1.08	245.00±2.00	154.67±1.17	174.00±1.00
P. longifolia (leaf)	Acetone	33.00±1.33	37.00±1.08	35.33±0.36	199.67±1.86	211.33±1.86	217.00±1.00
	Ethanol	38.00±0.63	35.00±1.67	32.00±1.00	235.50±1.03	203.50±1.07	200.00±1.08
	Hot water	36.10±0.33	30.00±0.08	35.37±1.36	209.6667±1.36	192.000±1.51	196.000±0.00
P. longifolia (bark)	Hot water	33.27±0.63	34.08±1.10	30.63±1.36	197.000±1.00	197.000±1.00	164.000±2.00
4-NQO	235.00±0.58				889.67±0.33		
10% DMSO	25.00±0.57				219.33±1.45		
Water	30.67±1.33				220.67±1.20		

4-NQO= 4-nitroquinoline-1-oxide, DMSO= dimethyl sulfoxide, *G. foliosa= Gerrardina foliosa*, *H. trifoliata= Heteromorpha trifoliata*, *H. colchicifolia= Hypoxis colchicifolia*, *P. acerosum= Phymaspermum acerosum*, *P. viridiflorum= Pittosporum viridiflorum*, *P. longifolia= Protorhus longifolia*, *P. echinatus= Pterocelastrus echinatus*

trus echinatus, and *Talinum caffrum*. Noteworthy antimycobacterial activity exhibited by the water extracts of *H. colchicifolia*, *H. trifoliata* and *P. acerosum* provided support for the traditional use of these plant species against TB in the eastern region of OR Tambo district. The antimycobacterial and safety findings demonstrated by crude extracts of some of the plants; *G. foliosa*, *H. colchicifolia*, *H. trifoliata*, *P. acerosum*, *P. echinatus* (leaf), and *P. longifolia* (bark), provide strong motivation for further investigation to determine if they could be sources of antimycobacterial drugs. However, cytotoxicity of extracts from bark of *P. echinatus* (acetone) and *P. longifolia* (ethanol and hot water) as well as genotoxicity of *P. viridiflorum* bark water and *H. trifoliata* leaf ethanol extracts suggest that they should be used with caution in traditional medicine. The findings of this study support suggesting the use of leaves of *P. viridiflorum*, *P. longifolia*, *P. echinatus*, and *Haemanthus albiflos* in traditional TB therapy as the leaf extracts also showed comparable antimycobacterial activity to the other more vulnerable parts of the plants, such as bulbs. Further studies should be aimed at determining the antimycobacterial activity against drug resistant *Mycobacterium* strains, the effect of combining active crude extracts with currently used anti-TB drugs, determining the intracellular antimycobacterial activity of active crude extracts in macrophages, and their effect on cytokine production.

List of Abbreviations

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 4-NQO = 4-nitroquinoline-1-oxide, ARVs = Antiretroviral drugs, BCG = Bacille Calmette–Guerin, DMSO = Dimethylsulfoxide, FCS = Foetal calf serum, FDA = Food and Drug Administration, HIV = Human Immunodeficiency Virus, INT = *p*-iodonitro-tetrazolium chloride, LC_{50} = Lethal concentration at which 50% at which cells are killed, MDR = Multi drug resistant, MEM = Minimal essential medium, MIC = Minimum inhibitory concentration, PBS = Phosphate buffered saline, TB = Tuberculosis, TDR = Totally drug resistant, WHO = World Health Organisation, XDR = Extensively drug resistant

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Conflict of interest

The authors declare no conflict of interest.

Author contribution list

BM conceptualised the study, conducted the research and wrote the manuscript. LM supervised the work, provided funding, edited and submitted the manuscript.

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