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Potency of extracts of selected plant species from Mbeere, Embu County-Kenya against *Mycobacterium tuberculosis*

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Tuberculosis is a serious chronic infectious disease affecting large global population. While efforts to control tuberculosis have intensified, they are challenged by rapid drug resistance development. For this reason, prospecting for compounds with potential antituberculous activity have been stepped up. The current study was done in a participatory appraisal manner to identify ten plants commonly used for management of "persistent coughs". Bioassays were conducted against *Mycobacterium tuberculosis* (H37Rv ATCC 27294) using the BACTEC MGIT™ 960 system. This was followed by assay of toxicity of the extracts towards Vero cells (ATCC CCL-81). Six extracts showed remarkable antitubercular activity. Four extracts had complete inhibition (0 GU- Growth Units) of *Mycobacterium tuberculosis*. The extracts were tested for their general antimicrobial activity and found to be broad spectrum antimicrobials. The highest activity against *Escherichia coli* (15.3 mm) was by *Cissampelos pareira,* while *Mangifera indica* yielded the highest activity against *Staphylococcus aureus* (11.7 mm) and *Candida albicans* (12.0 mm). In addition, six crude methanolic extracts were found to be within the acceptable toxicity limit (CC_{50}<90 µg/ml). The observed activity is attributable to phytochemicals in the extracts, including: phenols, terpenoids, flavonoids and anthraquinones. These findings could partly explain observed "positive" treatment outcome by indigenous people using these plant formulations.

Key words: Antibacterial activity, antituberculous activity, BACTEC MGIT™ 960 system, cytotoxicity, flavonoids, phytochemicals, terpenoids, Vero cells.

INTRODUCTION

Tuberculosis is a very serious chronic infectious disease affecting a large part of the population worldwide. Millions of people have died as a result of infection by the pathogen tubercle bacillus (Snider et al., 1994). Particularly of concern today is that the disease has spread to cover both developing and industrialized nations

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and this is accompanied by widespread emergence of drug-resistant strains of the pathogen. For example, the World Health Organization (WHO) estimates that, out of 9.6 million people who developed tuberculosis (TB) in 2014, 480,000 (5%) had multidrug resistant (MDR; resistance against isoniazid and rifampicin) (WHO, 2015). According to the WHO, the incidence of new tuberculosis infections have been steadily dropping (about 2% annually) over the last two decades when the disease was declared a global emergency (WHO, 2013). WHO TB Global Report (2015), also reported that, 9.6 million people are estimated to have fallen ill with TB in 2014 globally: 5.4 million men, 3.2 million women and 1.0 million children. 12% of the 9.6 million new TB cases in 2014 were also HIV-positive (WHO, 2015), highlighting the dangerous synergy between TB and HIV/AIDS.

In humans, tuberculosis is primarily caused by *Mycobacterium tuberculosis* (MTB) although to a lesser extent, other members of the *Mycobacterium* complex (*Mycobacterium bovis*, *Mycobacterium africanum*, and *Mycobacterium microti*) have been implicated in pathogenesis (Sreevatsan et al*.,* 1997). Without a known reservoir outside man, inhalation of aerosolized droplets containing infectious *M. tuberculosis* remains the predominant route of infection thus making pulmonary tuberculosis the prevalent form of infection (Glickman and Jacob, 2001). The tubercle bacilli are characterized by slow growth, dormancy, intracellular pathogenesis, genetic homogeneity, a complex cell envelope containing mycolic acid in their cell wall which makes them acid fast with a distinctively slow rate of division (~24 h) (Wheeler and Ratledge, 1994; Cole et al., 1998; Lawn and Zumla, 2011). These attributes enable it to persist in latent state for extensive periods of time, but perhaps more important accounts for the chronic phase of the disease. From a clinical perspective, the persistence imposes lengthy treatment regimens and presents a formidable obstacle to intervention (Cole et al*.,* 1998).

As a result of prolonged duration of therapy, there is the associated adverse toxicity as well as poor patient compliance to the treatment regimen. The poor compliance is often a cause for selection of drug resistant strains of tuberculosis which have been reported recently (multidrug resistant and extensively drug resistant tuberculosis) (Mariita et al., 2010a). The pathogen *M. tuberculosis* has been reported to rapidly develop resistance to several classes of antibiotics and this is largely attributable to it highly hydrophobic cell envelope which acts as a permeability barrier to most conventional drugs (Cole and Telenti, 1995). Additionally, other potential resistance determinants encoded in tuberculosis genome include: hydrolytic or drug modifying enzymes (beta-lactamases, aminoglycoside acetyl transferases) and many potential drug–efflux systems (14 members of the major facilitator family and numerous ABC transporters) (Brennan and Draper, 1994).

The aforementioned are some of the most important factors that has turned attention to tuberculosis necessitating continuous effort to counter its impact. This therefore means that several different classes and combinations of drugs continue to be developed and tested on tuberculosis. However, these efforts have further escalated cost of therapy. Continued attempts to scale this cost barrier have resulted in patients accessing alternative/traditional herbal therapy as they seek other options. It is believed that the answer to tuberculosis is hidden in the forest of the plant kingdom because this is one of the places where the untapped promise for treatment of infectious diseases lies. This is believed to be true especially in the context of developing countries where there is little or no access to modern health services (Mann et al., 2007; Idu et al., 2010). The current study builds upon previous efforts in prospecting for anti-tuberculosis activities among some traditional plants used in Eastern and Southern Africa (Tabuti et al., 2009; Earl et al., 2010; Mariita et al., 2010a). However, an important addition is that prior to conducting bioassays the main practitioners also known as herbalists were interrogated from whom identities of plants which have been used to manage “persistent coughs” was established. This was in an effort to contribute to prospecting efforts but perhaps more importantly to establish the safety of these plants. Therefore, an important prerequisite was the determination of toxicity levels of the plant extracts.

RESEARCH DESIGN

The current study employed both descriptive and laboratory research designs. The descriptive research was carried out in a participatory rural appraisal manner. In order to gather first hand data from the respondents (herbalists) which was helpful in establishing the identity of various medicinal plants used in the management of “persistent coughs” and other respiratory tract diseases. Thereafter, on the basis of information gathered, extraction and bioassays were conducted to determine the possible impact of the respective plant metabolites on *M. tuberculosis* and Vero cells.

Sample collection, preparation and phytochemical assay

Ten medicinal plants earlier identified by herbalists as useful in management of persistent coughs were used in this study (Table 1). None of these listed plants is an endangered species and were collected in open community field hence no prior permission was required. The geographical coordinates for the collection points were around 0°46'27.0"S 37°40'54.9"E; -0.774156 and 37.681908 (Kathuri village, Mbeere in Embu county, Kenya). These plants were later identified by a plant taxonomist in Egerton University (Njoro, Nakuru, Kenya) where voucher specimens were deposited and their numbers are recorded (Table 1). All plant parts were chopped into small pieces (about 2 to 3 cm) and air-dried under a shade at room temperature (23±2°C) to constant weight. The dry specimens were separately ground to powder in a mechanical grinder and separately macerated in methanol (50 g powder in 200 ml) for 48 h. Afterwards, the extracts were then filtered using a filter

Table 1. List of plants and parts used.

Botanical name	Family name	Voucher No.	Part(s) used
Aspilia pluriseta Schweinf.	Asteraceae	NSN2	Roots
Euphorbia ingens E.Mey. ex Boiss.	Euphorbiaceae	NSN3	Roots
Gnidia (Lasiosiphon) buchananii Gilg	Thymelaeaceae	NSN5	Roots
Mangifera indica L.	Anarcardiaceae	NSN6	Bark
Cissampelos pareira L.	Menispermaceae	NSN7	Roots
Dichrostachys cinerea (L.) Wight and Arn	Fabaceae	NSN8	Roots
Dalbergia melanoxylon Guill. & Perr.	Fabaceae	NSN9	Bark
Indigofera lupatana Baker F	Fabaceae	NSN1	Roots
Acacia ataxacantha DC	Fabaceae	NSN10	Roots
Lonchocarpus eriocalyx Harms	Fabaceae	NSN12	Barks

paper (Whatman's No. 1) and the filtrate concentrated *in vacuo* using a rotary evaporator (Büch Rotavapor R205, Switzerland), after which, products were allowed to air dry and their percentage yields recorded (Table 2). Once dry, the plant extracts were stored in air tight sample bottles at -20°C until next use. Standard procedures were employed for screening of the major classes of plant secondary metabolites in the extracts including: alkaloids, anthraquinones, terpenoids, phenolics and flavonoids (Harborne, 1984).

Antitubercular and antimicrobial activity screening

The test organism *M. tuberculosis* H37Rv ATCC 27294 was sourced from the Kenya Medical Research Institute (KEMRI), Nairobi. Prior to its use, the *M. tuberculosis* was revived on Lowenstein Jensen (LJ) slants for 14 days at 37°C following standard procedures (Gupta et al., 2010; Mariita et al., 2010a). The efficacy of the plant extracts against *M. tuberculosis* was carried out using the BACTEC MGIT 960 system. This is a fully automated, high volume, non-radiometric instrument that offers continuous monitoring of culture growth. The dry extract from each plant was first dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 1 g/ml. Growth supplement (0.8 ml) containing a mixture of oleic acid, bovine albumen, dextrose and catalase (OADC) was added to five 7 ml BBL™ MGIT™ tube labeled growth control, streptomycin, isonaizid, rifampicin, and ethambutol to provide essential substrates for rapid growth of *Mycobacteria*. 100 µl of BBL™ MGIT™ streptomycin, isonaizid, rifampicin, and ethambutol (SIRE) prepared aseptically according to the manufacturers' instruction was added to corresponding labeled BBL™ MGIT™ tube followed by addition of 0.5 ml of 1% *Mycobacterium* suspension. *Mycobacterium* suspension was prepared by pipetting 0.1 ml Middlebrook 7H9 Broth containing *Mycobacterium* adjusted to 0.5 McFarland standard into 10 ml sterile saline aseptically. The BACTEC MGIT™ 960 system was then loaded following the manufacturer's instructions and incubated at 37°C (Becton and Company, 2007). These served as the positive control (streptomycin at 1.0 µg/ml, isoniazid at 0.1 µg/ml, rifampicin at 1.0 µg/ml and ethambutol at 5.0 µg/ml whereas DMSO was used as a negative control). The procedure was repeated using plant extracts at 1.0 g/ml in place of SIRE. The general antimicrobial activity using *Escherichia coli* (ATCC 2592), *Staphylococcus aureus* (ATCC 25923) and *Candida albican* (ATCC 90028) was assayed by standard disc diffusion method according to Ayo et al. (2007), Mbaveng et al. (2008), Ngoci et al. (2012) and Mwitari et al. (2013).

Cytotoxicity screening

The toxicity of the plant extracts was assayed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay; a colorimetric assay based on the ability of mitochondrial enzyme (Succinate dehydrogenase) to reduce the yellow water soluble MTT into an insoluble colored compound called formazan (which can be measured Spectrophotometrically). Since only metabolically active cells can reduce MTT, the level of activity is usually directly proportional to the measure of the cell viability (Denizot and Lang, 1986). The test cell line used was Vero cells from African green Monkey Kidney cells (*Cercopithecus aethiops* epithelial cell line; ATCC CCL-81) (Mosmann, 1983). The cells were cultured in a T-75 flask containing Minimum Essential Medium (MEM) Eagle's Base supplemented with 15% Fetal Bovine Serum (FBS), 2.62 g/L $NaHCO_3$, 20 mM L-glutamine, 10 ml/L Penstrep 0.5 mg and Fungizoid. The cells were maintained at 37°C in 5% CO_2 until they attained confluency when they were harvested by trypsinization. Trypsin was inactivated within 1 min of action by addition of 8 ml of growth media and the cell crumps broken gently by sucking and releasing the cell suspension using a pipette. The harvested cells (2 ml) were then transferred into a 50 ml vial and topped up to 50 ml mark using growth media. Cell suspension (100 µl) at 1×10^5 cell/ml was seeded into two rows of wells A-H in a 96-well micro-titer plate for one sample. The cells were then incubated in 100 µl of MEM at 37°C and 5% CO_2 for 48 h to form a confluent monolayer. The growth medium was then aspirated off and replaced with 100 µl of maintenance medium. Afterwards, the Vero cells were exposed to increasing concentrations of respective plant extracts (from 2.0 to 500 µg/ml) and incubated at 37°C for 48 h. This was followed by a further incubation period of 4 h in 10 µl of 5 mg/ml MTT solution after aspirating off the plant extracts. Later, this was followed by addition of 100 µl acidified isopropanol (0.04 N HCl in isopropanol). The well plate was gently shaken for 5 min to dissolve the formazan and then Optical Density measured using ELISA Scanning Multiwell Spectrophotometer (Multiskan Ex labssystems) at 562 and 690 nm as reference. Rows of cells containing medium without plant extracts were included as negative control. Cell viability (%) was calculated at each concentration as follows (Ngeny et al., 2013).

$$\text{Cell viability (\%)} = \frac{OD_{\text{sample } 562} - OD_{690}}{OD_{\text{control } 562} - OD_{690}} \times 100$$

Statistical analysis

GraphPad Prism (version 6.04) and Ms Excel 2010 data sheet were

Table 2. Crude extract´s percentage yields and antituberculous activity.

Plants	% Yield	PC-GU	GC-GU	ME-GU	R/S	DH
Aspilia pluriseta	8	0	400	0	S	9.14
Euphorbia ingens	8	0	400	0	S	9.20
Gnidia (Lasiosiphon) buchananii	4	0	400	1	S	9.15
Mangifera indica	4	0	400	0	S	9.16
Cissampelos pareira	6	0	400	56.5	S	5.23
Dichrostachys cinerea	4	0	400	0	S	5.22
Dalbergia melanoxylon.	4	0	400	223	R	5.19
Indigofera lupatana	4	0	400	200	R	6.7
Acacia ataxacantha	4	0	400	400	R	8.4
Lonchocarpus eriocalyx	4	0	400	400	R	8.11

ME: Methanolic extract; GU: average growth units (n=2); R: Resistant; S: Sensitive; GC: Growth/negative control; PC: Positive control of streptomycin at 1.0 µg/ml, isonaizid at 0.5 µg/ml, rifampicin at 1.0 µg/ml and ethambutol at 5.0 µg/ml; DH: Days and Hours the BACTEC machine ran to yield results recorded.

used to analyze the data. The extract material from each plant was expressed as percentage yield and results presented in bar graph. Cytotoxicity using Vero cells was expressed as CC_{50} values, which is the concentration that kills 50% of the Vero cells. This was determined by Regression Analysis and results presented in bar graphs. A particular extract was considered cytotoxic if it had CC_{50} less than 90 µg/ml (Irungu et al., 2007). Unpaired t test was used to analyze antimicrobial activity. Diameters of zones of inhibition were expressed as mean±standard error of mean (SEM) and $p<0.05$ was used to test the level of significant difference between the test sample and the positive drug control.

RESULTS

The different crude extract yields are recorded and presented in (Table 2). The highest yield was 8% (4/50 g), while the lowest was 4% (2/50 g).

The interpretation of the antituberculous result data was based on the method previously described by Mariita et al. (2010b) and Lawson et al. (2013). When the growth unit (GU) of the growth control reached 400 (usually in 4 to 13 days), the GU values of the extract-containing vials were evaluated. If the GUs value of the extract-containing tube to be compared was ≥100, the strain was considered to be resistant (R) to the extract; while if the GU of the extract-containing tube was <100, the strains were considered to be sensitive (S) to the extract. Based on this, six plants crude methanol extracts were found to have considerable antituberculous activity. These were: (i) *Aspilia pluriseta*, (ii) *Euphorbia ingens*, (iii) *Gnidia buchananii*, (iv) *Mangifera indica*, (v) *Cissampelos pareira*, and (vi) *Dichrostachys cinerea*. Four of these extracts had similar GU as the positive control SIRE (Table 2). At the same time *Acacia ataxacantha* and *Lonchocarpus eriocalyx* extracts were inactive against MTB (Table 2), while another two, *Indigofera lupatana* and *Dalbergia melanoxylon* had an average sensitivity of 200 and 223 GU, respectively.

The extracts from *I. lupatana, A. pluriseta, G. buchananii*, and *M. indica* were cytotoxic having CC_{50} <90 (Table 3). While *I. lupatana* had no antituberculous activity, the other 3 plant extracts were active against *M. tuberculosis.* The rest of plant extracts were not cytotoxic indicating that they were tested within the acceptable toxicity limits. All plant extracts except *L. eriocalyx* had varying broad spectrum antimicrobial activity ranging from diameters of zones of inhibition of between 6.0 and 15.3 mm (Table 5). The activity was concentration dependent and the lowest MIC and MBC recorded was of 117 µg. The activity was either cidal or static as recorded (Table 5). However, there was significant difference ($P< 0.05$) between the activity of the test extract and the positive control drugs (Gentamycin, Oxacillin and Nystatin).

Phytochemical results demonstrated that all plant extracts had phenols and terpenoids in varying quantities, while flavonoids were found to be present in three plant extracts. Anthraquenones were found only on one plant extract while alkaloids were absent in all extracts (Table 4).

DISCUSSION

A participatory approach was used to identify medicinal plants for use in this study. While there have been previous studies undertaken on antibacterial activities on some of these plants used in the current study (Khalil, 2003; Abubakar, 2009; Sripathi and Sankari, 2010; Aworet-Samseny et al., 2011; Ighodaro et al., 2012), to the best of our knowledge, there is little scientific information to confirm their antituberculous activity. Though Cateni et al. (2003) hypothesized about antimycobacterial activity of *Euphorbia* species and *C. pareira* has been implicated by Antoun et al. (2001) as having antituberculous activity, no proper antituberculous

Table 3. Cytotoxicity results of methanolic extracts.

Plant	CC_{50} (µg/ml)
Aspilia pluriseta	24.51
Euphorbia ingens	105.55
Gnidia (Lasiosiphon) buchananii	76.24
Mangifera indica	88.61
Cissampelos pareira	179.02
Dichrostachys cinerea	201.22
Dalbergia melanoxylon.	120.04
Indigofera lupatana	60.37
Acacia ataxacantha	90.39
Lonchocarpus eriocalyx	201.87

CC_{50}: Concentration that kills 50% of the cells. The plant extracts were two-fold serial diluted to varying concentrations ranging from 3.90625 to 500 µg/ml. CC50 values ≤90 µg/ml were considered to be cytotoxic (Irungu et al., 2007).

Table 4. Phytochemical tests.

Plant	Test for				
	Phenols	Terpenoids	Flavonoids	Anthraquinones	Alkaloids
Aspilia pluriseta	+	+++	+	-	-
Euphorbia ingens	+	++	-	-	-
Gnidia (Lasiosiphon) buchananii	+	+	-	-	-
Mangifera indica	+	++	-	-	-
Cissampelos pareira	+	++	++	+	-
Dichrostachys cinerea	+	+	-	-	-
Dalbergia melanoxylon.	+	++	-	-	-
Indigofera lupatana	+	+	-	-	-
Acacia ataxacantha	+	++	-	-	-
Lonchocarpus eriocalyx	++	+	+	-	-

+, Low concentration of phytochemicals; ++, Medium concentration; +++, High concentration of phytochemicals and -, Absence of phytochemicals.

studies on the target plants has been undertaken using the pathogenic MTB strain.

Cytotoxicity of plant extracts is crucial in determining safety, particularly in the context of TB therapy that often entails lengthy treatment regime (Zaleskis, 2006). A major output of the current study is the identification of six plants methanolic crude extract that demonstrate antituberculous activity with accompanying data on their toxicity levels showing that the used concentration was still within the acceptable toxicity margin. This is particularly important as these extracts become promising candidates for further testing in intracellular assays. However, even the other tested extracts (Table 3) that show cytotoxicity should not be disqualified as drug candidates as structural modification can be undertaken to improve on their safety. They can also be looked upon as possible candidates for cancer treatment (Ngeny et al., 2013).

In addition to antituberculous activity, the plant extracts had broad spectrum activity as they inhibited growth of Gram positive, Gram negative bacteria and a fungus. The inhibition zones were dose dependent (Figure 1). Variation was observed with the microbial strain tested indicating selectivity in the activity of the extracts. Gram positive strain (*S. aureus*) was more susceptible to the extract often yielding higher zones of inhibition than Gram negative (*E. coli*) (Table 5) and fungal strain (*C. albicans*). This corroborates previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Parekh and Chanda, 2006; Mohamed et al., 2010; Ngoci et al., 2012). The higher sensitivity of Gram-positive bacteria has been attributed to their outer peptidoglycan layer which is not an effective permeability barrier as compared to the outer phospholipid membranes of Gram-negative bacteria (Trombetta et al., 2005; Tomczykowa et al., 2008; Kaur and Arora, 2009;

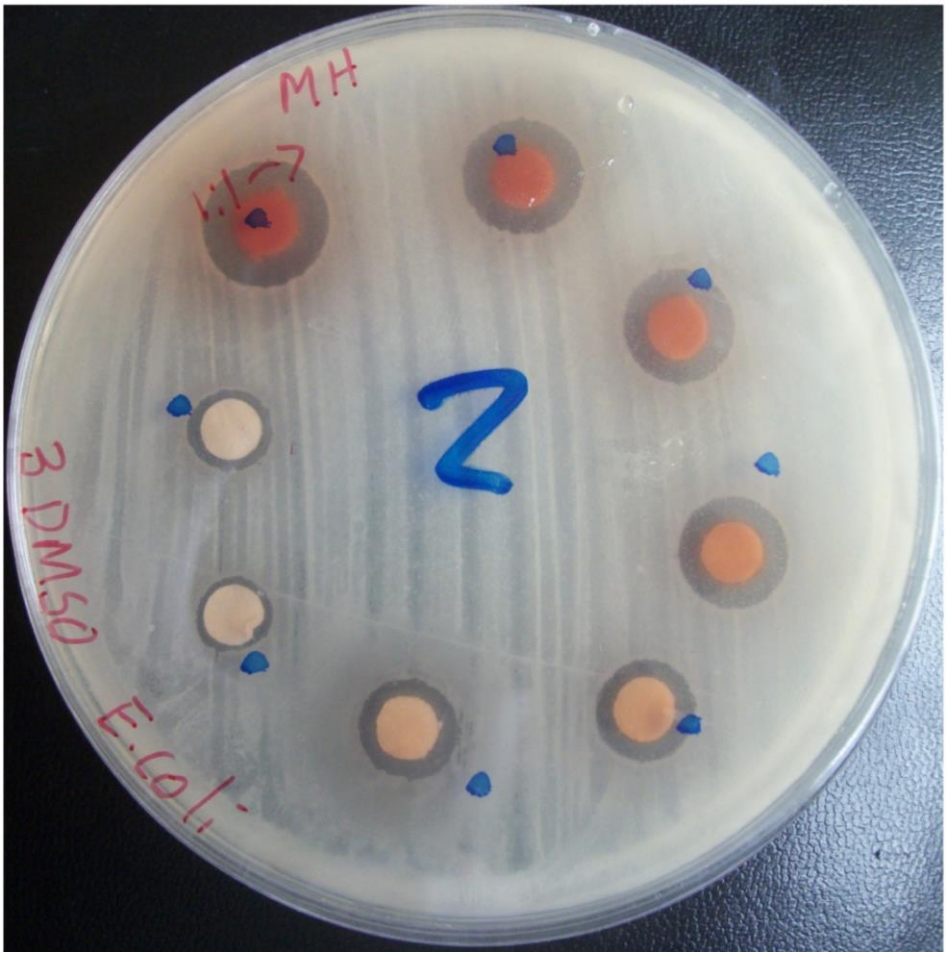

Figure 1. Zones of inhibition of *Mangifera indica* methanolic extract against *E. coli*. The extract was serially diluted and zones of inhibition diameter decreased as concentration decreased demonstrating a concentration dependent activity.

Ngoci et al., 2012).

Although the concentrations of the extract fractions were in the range of 100 times more than the standard drugs (positive controls in both antituberculous and general antimicrobial sensitivity testing), they showed marked anti-microbial activity as evidenced by their zones of inhibition and zero GU. This could be due to the fact that the active components in the extract comprise only a fraction of the total extract used. Therefore, the concentration of the active components in the extract could be much lower than the standard antibiotic used. It is important to note: if the active components were isolated and purified, they would probably show higher antibacterial activity than those observed in this study.

The activity observed in the plant extracts in the current study may be associated with the group of phytochemical compositions tested in the extracts. For example, the current study established the presence of terpenoids in all extracts in varying concentrations and flavonoids in *A. pluriseta* and *C. pareira*. Other studies have shown that flavonoids have antituberculous activity and they function mechanistically by inhibiting *de novo* fatty acid biosynthesis in *Mycobacteria*, inhibiting mycolic acid biosynthesis, proteasome inhibition, topoisomerase inhibition, inhibition of phosphatidyl-inositol 3-kinase, induction of cell cycle arrests, accumulation of p53 or enhanced expression of c-fos and c-myc genes (Yuan et al., 2009; Mariita et al., 2010b). Flavonoids have also been shown to have antimicrobial activity and to act by complexing microbial proteins and disrupting microbial membranes (Cowan, 1999; 2003; Al-Bayati and Al-Mola, 2008; Samy and Gopalakrishnakone, 2008; Kaur and Arora, 2009; Ngoci et al., 2011, 2012). On the other hand, terpenoids have been shown to have antibacterial activity (Cowan, 1999), although the mechanism of action is not well understood but it is thought to involve membrane disruption and inhibition of protein synthesis (Cowan, 1999; Mariita et al., 2010b). *C. pareira* was found to have terpenoids, flavonoids and anthraquinones and this is in agreement with earlier study by Ngoci et al.

Table 5. Antimicrobial activity.

Plant	ZID in mm at (15×10^3 µg)			MIC (µg)			MBC (µg)			Effect (BS/BC)		
	E.C	S.A	C. A	E.C	S.A	C. A	E.C	S.A	C. A	E.C	S.A	C. A
Aspilia pluriseta Schweinf.	11.6±1.2	9.0±0.6	7.7±0.3	234	1875	3750	>15000	1875	3750	BS	BC	BC
Euphorbia ingens	11±1.7	10±0.9	11.7±0.3	234	234	117	>15000	7500	117	BS	BC	BC
Gnidia (Lasiosiphon) buchananii	6.7±0.3	7.3±0.3	6.7±0.3	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mangifera indica	11.0±0	11.7±0.3	12.0±0.6	234	117	1875	468	117	1875	BS	BC	BC
Cissampelos pareira	15.3±0.3	11.0±1.0	11.7±0.3	234	117	468	>15000	117	468	BS	BC	BC
Dichrostachys cinerea	6.0±0	6.0±0	6.0±0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dalbergai melanoxylon.	12.0±0.6	11.0±1.0	11.7±0.3	117	117	468	>15000	>15000	937	BS	BS	BS
Indigofera lupatana	7.0±0	6.3±0.3	6.7±0.3	NA	NA	NA	NA	NA	NA	NA	NA	NA
Acacia ataxacantha	8.3±0.7	6.7±0.3	7.0±0.6	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lonchocarpus eriocalyx	6.0±0	6.0±0	6.0±0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Positive standard	22.0±0	24.7±1.3	16.3±0.9	NT	NT	NT	NT	NT	NT	BC	BC	BC
Negative Control	6.0±0	6.0±0	6.0±0	NA	NA	NA	NA	NA	NA	NA	NA	NA

ZID: Zone of inhibition diameter taken in mm (mean±SEM, n=3); MIC: minimum inhibitory concentration; MBC; minimum bactericidal concentration; EC: *E. coli*; SA: *S. aureus*; CA: *C. albican*; BS: bacteriostatic effect; BC: bactericidal effect; NA: not applicable; No inhibition zone observed; NT: not tested; Positive Standard: Gentamycin (10 µg) for *E. coli*, Oxacillin (10 µg) for *S. aureus* and Nystatin (100 µg) for *C. albican*; Negative control: A disc loaded with 15 µl of DMSO. Unpaired t test analysis demonstrated that there was significant difference in zones of inhibition ($P< 0.05$) between the test sample mean and positive control drug means.

(2014), though alkaloids that tested positive then, tested negative in this particular study. Phytochemical differences on what other scientist have published and what we tested from same plant can be associated with the great diversity of plants bioactive compounds. This diversity of bioactive compounds from same plant species is influenced by; genetic characteristics, environmental factors such as climate, altitude and soil type; the period when collection took place, the treatment after collection and existence of a distinct phenotype of a particular species (also known as chemical races). This diversity can either be in regard to presence and absence of certain phytochemicals or be in the levels of concentration of a certain phytochemical in a plant sample. Therefore, the observed activity in the current study could also be attributed to the presence of flavonoids and terpenoids which have been shown to have capacity to traverse the highly hydrophobic tubercle envelop (Edwards and Ericsson, 1999; Rao et al., 2010).

Conclusion

Natural products are proven templates for the development of new arsenals of drugs for fighting and management of various diseases, and therefore, they have received considerable attention as potential alternative anti-tuberculosis agents. Our findings preliminarily demonstrate that methanolic extracts of 6 indigenous plants which we worked on had potential in management of tuberculosis while virtually all extracts had varying antimicrobial activity. This demonstrated that there is a degree of reliability in the traditional systems which lead to the identification of these plants. Indeed more work is needed, some of which is already underway to fractionate the plant extracts and possibly identify the specific active components, with a view of deciphering their mode(s) of action.

Conflict of Interests

The authors have not declared any conflict of interests.

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