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Sospeter Ngoci Njeru
Department of Medicine, Kisii University, PO Box, 408-40200, Kisii-Kenya.

Meshack A. Obonyo
Department of Biochemistry & Molecular Biology, Egerton University, P.O Box. 536-20155, Egerton.

Samwel O. Nyambati
Department of Biochemistry & Molecular Biology, Egerton University, P.O Box. 536-20155, Egerton.

Silas M. Ngari
Department of Chemistry, Egerton University, P.O Box. 536-20155, Egerton.

Correspondence:
Sospeter Ngoci Njeru
Department of Medicine, Kisii University, PO Box, 408-40200, Kisii-Kenya.

Bioactivity of *Cissampelos pareira* medicinal plant against *Mycobacterium tuberculosis*

Sospeter Ngoci Njeru, Meshack A. Obonyo, Samwel O. Nyambati, Silas M. Ngari

Abstract
Tuberculosis is ranked as leading cause of death from a single micro-organism. Studies aimed at identifying alternative drugs for managing tuberculosis need to be considered. Medicinal plants are hoped to fill this gap. Therefore, our study was aimed at answering the question of whether *Cissampelos pareira* has anti tuberculosis activity. This was addressed using *in vitro* and chemical tests. We found out that, antituberculous activity was very high with MIC ranging between 50 to 6.25 µg/ml among all fractions. General antibacterial activity was broad spectrum with methanolic fraction yielding the highest activity of 28 mm in *S. aureus* and the lowest MIC of between 31.25 and 62.5 µg/ml and the fraction was not toxic. The activity could be due to phytochemicals alkaloids, flavonoids, terpenoids, anthraquinones and phenols present in extract fractions. We therefore suggest that methanolic fraction provide a viable candidate for novel safe antimicrobial drugs.

Keywords: Antituberculous activity, antimicrobial activity, herbal medicine, Phytochemicals, Cytotoxicity.

1. Introduction
Infectious diseases are the world's main cause of human and animal mortality. This is further aggravated by the rapid development of multi-drug resistance to available anti-microbial agents [1], limited anti-microbial therapy spectrum, anti-microbial side effects, and emergence and re-emergence of opportunistic infections [2]. One of the major global health problem is attributable to diseases, such as tuberculosis (TB). Tuberculosis is one of the oldest and most pervasive diseases in history. It is a bacterial infectious disease caused by *Mycobacterium tuberculosis* (MTB) and to some lesser extent by *M. bovis* and *M. africanum* [3, 4]. MTB are obligate, intracellular, aerobic, non-motile, non-sporing and non-capsulated bacilli. They are acid-fast due to presence of mycolic acid in their cell wall. They divide every 15–20 hours, which is extremely slow compared with other bacteria, for example, *Escherichia coli* divides every 20 minutes. This slow replication rate and ability to persist in latent state results in a need for a long duration of drug therapy thus creating poor patient compliance [5]. Non-compliance is a frequent cause for selection of drug resistance and often, the deadly multidrug resistant TB (MDRTB) bacteria, and recently, extensively drug resistant tuberculosis (XDR-TB) [6]. This is coupled with the problem of drug toxicity and mycobacterial persistence, thus highlighting the need to develop novel TB drugs that are not only active against drug resistant bacteria, but more importantly, kill persistent bacteria and shorten the length of treatment [6, 7].
Tuberculosis is ranked as leading cause of death due to a single infectious organism. Approximately one third of the world is infected with MTB, with 8.9 to 9.9 million new and relapse cases of TB and 2 million deaths being reported every year [8, 9]. The health burden of TB is further complicated by the rapid development of resistance towards conventional drugs with 440 000 new cases of MDR-TB being reported and accounting for at least 150 000 deaths annually. This is aggravated by the existence of strains that are tolerant to multiple drug regiments and the adverse effects of the first- and the second–line antituberculous drugs. This scenario in Kenya is further complicated by prevalence of TB among resource poor part of the population who unfortunately are unable to bear the cost of TB treatment. TB is also a major opportunistic infection in Africa among patients suffering from HIV/AIDS. Studies that are directed at identifying alternative formulations that hold the potential for being used in managing this disease deserve attention. Medicinal plants have recently received increased research interest in hope of filling the gap by discovering new antitubercular leads. Although herbal plants provide the cheap alternative to conventional drugs, their therapeutic information

is only folkloric based and thence there is need to undertake their scientific rationalization through *in vitro* and *in vivo* testing [8, 9].

In this study we focused our attention in unearthing the antitubercular activity of *Cissampelos pareira*. *Cissampelos pareira* L. (Menispermaceae), locally in Mbeere community of Embu County in Kenya called '*Karigi – kanonongwe'* [10] is a woody, climbing ground creeper vine. It produces inedible, dark, grape-sized drupes of about 4 – 6 mm. It is also distributed throughout warm parts of Asia, East Africa and America. The roots are used as a diuretic and febrifuge, as a remedy for heart trouble, dysentery and sores. The roots of this plant are mainly incorporated into many traditional Ayurvedic formulation prescribed to manage diseases like rheumatism, ulcers, fevers, cough, intestinal worms, diarrhoea, dysentery, menstrual and pregnancy complications, stomachic, wounds, snake bite, colics, urogenital problems, jaundice, urogenital problems and in being an antiperidic [10-13].

2. Methods

2.1 Plant material

The plant for this study was identified through ethnobotanical approach. The information of its use and preparation in Mbeere community, Kenya was gleaned from local herbalist and confirmed from documentation by Relay and Brokensha [10] in *The Mbeere in Kenya (ii), Botanical identity and use*. This plant is not an endangered species and it was collected in open community field and therefore no prior permission was required. The location for collection was around 0°46'27.0"S 37°40'54.9"E; -0.774156, 37.681908 of GPS co-ordinates. The identity was also confirmed by a Botanist at Egerton University where voucher specimen number NSN7 was given and samples deposited with the University herbarium.

2.2 Plant Extract preparation

Root samples were chopped into small pieces of 2-3 cm and air-dried in dark at room temperature (23±2 °C) to constant weight. Using a mechanical grinder, the dried root specimens were ground to powder. Fractionation of powdered root part of *C. pareira* was done using different solvents of increasing polarity. The root powder (50 g) was macerated in 200 ml of Petro ether with intermittent shaking for 48 hours after which it was filtered using Whatman no 1 filter paper. The residue was further re-extracted using the same fresh solvent for 24 hours and thereafter the filtrates pooled together. The resulting residue was air dried and further extracted with Dichloromethane followed by Ethyl acetate and lastly methanol using the same procedure carried out for Petroleum ether. Using a rotary evaporator, the solvent was removed from each filtrate under conditions of reduced temperature and pressure. The resulting dry extract was weighed and stored in air tight sample bottles at -20 °C until next use.

2.3 Culturing of Micro organisms

One Gram positive; *Staphylococcus aureus* (ATTC 25923) strain and Methicillin Resistant *Staphylococcus aureus* strain (clinical isolate), five Gram negative; *Escherichia coli* (ATTC 25922), *Klebsiella pneumoniae* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (clinical isolate) and *Shigella sonnei* (clinical isolate) and two Fungi; *Candida albicans* (ATTC 90028) and *Cryptococcus neoformans* (ATTC 66031) were used to investigate for general antimicrobial activity. These organisms were sourced from Kenya Medical Research Institute (KEMRI) - Nairobi. The extracts were also tested against acid fast *Mycobacterium tuberculosis* strain H37Rv (ATCC 27294) [14, 15].

2.4 Disc diffusion test

The antibacterial activity was assayed by disc diffusion method according to CLSI [16] and Mbaveng *et al.,* [17] with slight modifications. Fresh inoculum was prepared by suspending activated colonies in physiological saline water (0.85% NaCl). Using 0.5 McFarland turbidity standard, the bacteria and fungi suspensions were adjusted to 1.5 x 10^6 CFU/ml after which they were inoculated aseptically by swapping the surfaces of the Muller Hinton (MHA) plates and SDA plates. Whatmann filter paper (No.1) discs of 6 mm diameter were made by punching the paper, and the blank discs sterilized in the hot air oven at 160 °C for one hour. They were then impregnated with 10 µl of various stock extract solution. The petro ether, dichloromethane, and methanol fractions stock solutions were made at 500 µg/ml while ethyl acetate at 250 µg /ml. This afforded disc extract concentration of 5 µg/disc for petro ether, dichloromethane, and methanol fractions and 2.5 µg/disc for ethyl acetate. Three standard drugs were used as positive controls: Oxacillin 10 µg/disc (Oxoid Ltd) and Gentamycin 10 µg/disc (Oxoid Ltd) for Gram positive and Gram negative bacteria respectively. Nystatin 100 µg/disc (Oxoid Ltd) was used as the standard drug for all fungi while discs loaded with 10 µl of DMSO was used as negative controls. Using a sterile forceps, the impregnated dry discs were carefully placed on the agar plates at equidistance points. A positive control as well as a negative control was incorporated in each plate and the plates incubated at 4 °C for 2 hours so as to allow the extract pre-diffusion into the media after which they were incubated at 37 °C for 18 hours. Antimicrobial activity was determined by measuring the size of the inhibition zone to the nearest mm and the results recorded. Extracts fractions that gave an inhibition zone of more than 10 mm were considered to be active [16] and therefore their MIC (Minimum inhibitory concentration) and MBC (Minimum bactericidal concentration) determined [18].

2.5 Determination of MIC and MBC

The MIC and MBC of the plant *C. pareira* extracts was determined for all the organisms in triplicates using broth micro-dilution assay. The petro ether, dichloromethane, and methanol fractions stock solutions were made at 500 µg/ml while ethyl acetate at 250 µg/ml with DMSO. To 100 µl of nutrient broth agar in a sterile 96 well plate, 50 µl of varying plant concentration (petro ether, dichloromethane, and methanol fractions at 500 to 3.91 µg/ml while ethyl acetate at 250 to 1.95 µg/m) was added followed by 50 µl of test organisms previously diluted to equivalent of 0.5 McFarland standard. Addition of the test organisms was done in all the wells except for wells of column 11 which contained neat DMSO and broth, this served as control to check for purity. The adequacy of the media to support the growth of the test organism was evaluated by putting the broth and the test organism in wells of column 12. The plates were then covered with a sterile "cling-on" sealer and incubated for 24 hrs at 37 °C. Bacterial growth was evaluated by addition of 40 µl of 0.2 mg/ml p-iodonitroterazolium chloride (INT, Sigma) to each well and incubated for 30 min. Growth of bacteria was detected by formation of a pink-red coloration while inhibition of growth was signaled by persistence of a clear coloration. The lowest concentration that exhibited color change was considered as the MIC. MBC was determined by streaking a

loopful of broth from wells that exhibited no color change onto sterile nutrient agar and sabouraud dextrose agar for bacteria and fungi respectively and thereafter incubated at 37 °C for 24 hrs. The lowest concentration that exhibited no growth was considered as the MBC [19].

2.6 Antitubercular activity

The test organism *Mycobacterium tuberculosis* H37Rv ATCC 27294 was sourced from the Kenya Medical Research Institute (KEMRI), Nairobi. Prior to its use, the *Mycobacterium tuberculosis* was revived on Lowenstein Jensen (LJ) slants for 14 days at 37 °C following standard procedures [14, 15]. 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. Growth supplement (0.8 ml) containing a mixture of OADC- Oleic Acid, Bovine Albumen, Dextrose and Catalase was added to five 7 ml BBL™ MGIT™ tube labeled GC (growth control), STR (streptomycin), INH (isonaizid), RIF (rifampicin), EMB (ethambutol) to provide essential substrates for rapid growth of *Mycobacteria*. 100 µl of BBL™ MGIT™ SIRE (streptomycin, isonaizid, rifampicin, ethambutol) 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 [20]. 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 petro ether, dichloromethane, ethyl acetate and methanol solvent fractions. The fractions were tested at concentrations ranging from 50 to 6.25 µg/ml (petro ether, dichloromethane and methanol) or 25 to 3.125 µg/ml (ethyl acetate) to determine the MIC.

2.7 Cytotoxicity Screening

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay was used to determine the toxicity of the extracts obtained from the plant. This is a colorimetric assay hinged on the ability of mitochondrial enzyme (Succinate Dehydrogenase) to reduce yellow water soluble MTT to an insoluble colored substance (formazan) that is spectrophotometrically measurable. The level of formazan is directly proportional to the measure of cell viability because only metabolically active cells can reduce MTT. Test cell line used was Vero cells from African green Monkey Kidney cells (*Cercopithecus aethiops* epithelial cell line; ATCC CCL-81). The test cells were grown in growth media comprising of 100 ml DMEM, 10 ml Fetal Bovine Serum (FBS), 1 ml Penstrep, 1 ml Amphotericin B, 1 ml L Glutamine. The test cells were incubated at 37 °C in 5 % CO_2 until they attained confluency after which they were passaged by adding 2 ml of 0.25 % trypsin and further incubated at room temperature until the cell were detached. Growth media (6 ml) was introduced to the test cells to inactivate trypsin off action, while the cell crumps formed were broken gently by sucking and releasing the cell suspension using a pipette. Two ml of the harvested cells were then transferred into a 50 ml vial and topped up to 50 ml mark using growth media. A cell suspension of 100 µl (1 x 10^5 cell/ml) was seeded into two rows of wells A-H in a 96-well micro-titer plate for one sample. The test cells were then incubated in 100 µl growth media at 37 °C and 5% CO_2 for 48 hrs to form a confluent monolayer. The growth medium was then aspirated-off and replaced with 100 µl of maintenance. Afterwards, cells were exposed to increasing concentrations of respective plant extracts (from 1.95 µg/ml to 500 µg/ml) and incubated at 37 °C for 48 hrs. This was followed by a further incubation period of 4 hours in 10 µl of 5 mg/ml MTT solution after aspirating off the plant extracts. This was followed by addition of 100 µl acidified isopropanol (0.04 N HCl in isopropanol). The well plate was gently shaken for 5 minutes to dissolve the formazan and then optical density measured using ELISA Scanning Multiwell Spectrophotometer (Multiskan Ex labssystems) at 562 nm and 690 nm as reference. Rows of cells containing medium without plant extracts were included to act as negative control. Cell viability (%) was calculated at each concentration as follows using the formula [21].

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

2.8 Phytochemical Tests

Phytochemical tests were done to determine the classes of compounds present in the active fractions that could be responsible for activity and/or cytotoxicity. They were identified by characteristic colour changes based on standard procedures according to [22-24]. The results were reported as (+) for presence, and (-) for absence.

2.9 Alkaloids

Six to eight drops of Dragendorff reagent was mixed with 2 ml of the extract. Formation of brownish-red precipitate indicated presence of alkaloids. The Dragendorff reagent was prepared by mixing two reagents: reagent 1 and reagent 2 in equal parts. Reagent 1 was made by dissolving 8.5 g of Bismuth subnitrate in a solution of 10 ml acetic acid and 40 ml of distilled water while as Reagent 2 was prepared by dissolving 8 g of potassium iodide in 20 ml of water [25-26].

2.10 Phenols

Phenols were detected using ferric ferichloride which was prepared by dissolving 0.1 g of ferric ferichloride in 10 ml of water. Equal volumes (2 ml) of both ferric ferichoride and the plant extract were mixed. Formation of a violet- blue color or greenish color was evidence that phenols presences [25-26].

2.11 Terpenoids

1 gram of Vanillin was mixed with 100 ml of concentrated sulphuric acid after which 2 mls of the resultant solution was mixed with 2 mls of the plant extract. Formation of a blue-green ring or pink- purple coloration signified presences of terpenoids [25-26].

2.12 Anthraquinones

0.5 ml of the plant extract was mixed with 0. 5 ml of 10% methanolic potasium hydroxide. Red coloration indicated presences of anthroquinones. 10% methanolic potassium hydroxide was prepared by dissolving 0.5 g of potassium hydroxide pellets in 50 mls of methanol [25-26].

2.13 Flavonoids

5 ml of dilute aqueous ammonia solution was added to a portion of the aqueous filtrate of the plant extract, followed by concentrated sulphuric acid. A positive test result was confirmed by the formation of a yellow coloration that disappeared instantly [23-24].

2.14 Statistical analysis

Ms Excel 2010 data sheets and Graphpad Prism version 6 were used to analyze the data. The data on cytotoxicity was expressed as a percentage of the untreated controls. CC_{50} values, which is the concentration that kills 50% of the Vero cells, was determined by Regression Analysis. A particular fraction's extract was considered cytotoxic if it had CC_{50} of less than 90 μg/ml [27]. Furthermore, unpaired student's t-test was used to test for statistical significance in the differences between the treatments and the control in this study. A *p* value of less than 0.05 was considered to indicate statistical significance. Values were expressed as mean ±S.E.M.

3. Results and Discussion

Various ethnopharmacologic studies carried out throughout Africa have confirmed that local plants are the main constituents of traditional African medicine [28]. There is renewed interest in this area of ethnopharmacology because much valuable knowledge is being lost with urbanization and westernization of many aspects of life. Herbal remedies that have been validated scientifically will help reduce the burden of debt incurred by the import of expensive conventional pharmaceuticals [22]. Traditional healers use medicinal plants for a variety of illness such as chest pain, tuberculosis, malaria, diarrhea, asthma, bronchitis, pneumonia among others [29]. A few of these plants have been scientifically investigated and their valuable products are in clinical trial stage [30]. However majority have not been studied and to the best of our knowledge, this is the case with our target *C. pareira* plant in regard to its antituberculous activity and therefore this was the driving force for the current study.

The information on use and preparation of *C. pareira* plant in Mbeere community, Kenya was gleaned from tradipractitioners and herbalist and confirmed from documentation by Relay and Brokensha [22]. The plant has been used by community members mainly for management of respiratory related illnesses. In other communities in the world, it is used to cure gastrointestinal complaints such as diarrhea, dysentery, ulcers, colic, intestinal worms and digestive complaints, and also urogenital problems such as menstrual problems, venereal diseases, infertility, uterine bleeding, and threatening miscarriage among others [13]. Our earlier work with crude methanolic extract at a concentration of 1 g/ml shown activity against MTB with average growth units (GU) of 56.5 (data not shown). All the fractions tested in this experiment had very high antituberculous activity with MIC ranging between 50 to 6.25 μg/ml considering the threshold MIC level of 100 μg/ml [31]). Therefore, we identify *C. pareira* as a very strong candidates to be tapped for novel drug lead for management or/and treatment of TB. This is supported by our data showing that the level of inhibition (GU- 0) by drug standards (SIRE) is same as that achieved by some of the tested concentrations (Table 1).

Table 1: Antituberculous results for *C. pareira* fractions

Plant	Fraction	Antituberculous testing Concentration μg/ml	GU	R/S	MIC (μg/ml)
C. pareira	PE	50	0	S	
		25	400	S	
		12.5	400	R	50
		NC	400	R	
		SIRE	0	S	
	DCM	50	NT	-	
		25	NT	-	NT
		12.5	NT	-	
		NC	NT	-	
		SIRE	0	S	
	EA	25	0	S	
		12.5	0	S	
		6.25	0	S	6.25
		NC	400	R	
		SIRE	0	S	
	MOH	50	0	S	
		25	0	S	12.5
		12.5	18	S	
		NC	400	R	
		SIRE	0	S	

PE: Petro ether fraction; DCM: Dichloromethane fraction; EA: Ethyl acetate fraction; MOH: Methanol fraction; SIRE: 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; GU: Growth unit; NC: Negative control of media treated with DMSO; R: Resistant; S: Sensitive; NT: Not tested.

We also screened each fraction for general antimicrobial activities and the fractions exhibited broad spectrum activity. Each fraction gave varied antimicrobial activity and this difference indicates that the root part of *C. pareira* contains several antibacterial and antifungal compounds of different polarities as supported by phytochemical studies (table 5). Additionally, sensitivity variance with micro-organism tested could be due to genetic differences between different strains. This proofs the necessity of antibiogram prior to prescription as a precautionary measure in mitigating drug resistance development [32]. The highest activity was by methanolic extract against *S. aureus* with inhibition zone of 28±1.2 mm

and dichloromethane extract fraction against *S. aureus* (24.3±0.3). There was a resistance by all fractions against *P. aeruginosa*, *K. pneumonia* and *S. typi* with average inhibition zones of 6.0 mm. interestingly, all fractions had higher activity against *S. aureus*, demonstrating the potential of the plant to be used in curing diseases caused by this organism. The general activity against Gram positive bacteria was higher than Gram negative strains and fungi. This is in agreement with previous studies that plant extracts are more active against Gram positive bacteria than Gram negative bacteria. The higher sensitivity of Gram-positive bacteria could be attributed to their cell wall peptidoglycan layer that is not an effective permeability barrier as compared to the outer phospholipid membranes of Gram-negative bacteria [33-35].

Table 2: Antimicrobial activity for *C. pareira* fractions

Diameter of zones of inhibition for the fractions in mm									
Fraction	Gram positive		Gram negative					**Fungi**	
	SA	MRSA	PA	EC	KP	SH	ST	CA	CR
PE	18.3±0.9	ND	6±0	10.3±0.3	6±0	6±0	6±0	9.3±0.6	ND
DCM	24.3±0.3	15.0±0.6	6±0	14.7±0.3	6±0	10±0.6	6±0	12.7±0.3	6±0
EA	23.7±0.3	6±0	6±0	6±0	ND	6±0	6±0	6.3±0.3	6±0
MOH	28±1.2	16.3±0.3	6±0	13.7±0.3	6±0	11.7±0.7	6.7±0.3	12.7±0.3	ND
PC	33.7±0.3	24.3±0.3	23.7±0.6	17±0.6	15.7±0.3	19.7±0.6	21.3±0.3	16.3±0.3	20.3±0.3
NC	0	0	0	0	0	0	0	0	0

PE: Petro ether fraction at 5 µg/disc; DCM: Dichloromethane fraction at 5 µg/disc; EA: Ethyl acetate fraction at 2.5 µg/disc; MOH: Methanol fraction at 5 µg/disc; PA: *P. aeruginosa*; EC: *E. coli*; SA: *S. aureus*; KP: *K. pneumoniae*; MRSA: *Methicillin Resistant S. aureus*; SH: *Shigella*; ST: *S. typhi*; CA: *C. albicans*; CR: *Cryptococcus*; PC: Positive control (Oxacillin 10 µg/disc and Gentamycin 10 µg/disc for Gram positive and Gram negative bacteria respectively. Nystatin 100 µg/disc for fungi); NC: Negative control (Discs loaded with 10 µl of DMSO); n=3; values= Mean±SEM.

Notably, the lowest MIC of 31.25 µg/ml was recorded in petro ether against *E. coli*, dichloromethane extract against MRSA and methanolic fraction against *S. aureus,* MRSA and *C. albican* which is less than 100 µg/ml; the set threshold for plant extract (table 3) [31].

Table 3: MIC and MBC results for the *C. Pareira* Fractions

MIC and MBC for the Fractions (µg/ml)										
	Gram Positive		**Gram Negative**						**Fungi**	
	SA		**MRSA**		**EC**		**SH**		**CA**	
Fraction	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Petro ether	125	-	-	-	31.25	-	-	-	-	-
Dichloromethane	-	-	31.25	-	62.5	500	-	-	-	-
Methanol	31.25	-	31.25	-	62.5	-	62.5	-	62.5	31.25

MIC: Minimum Inhibitory Concentration; MBC; Minimum Bactericidal Concentration; BS: Bacteriostatic effect; **-**: >500 µg/ml; EC: *E. coli*; SA: *S. aureus*; MRSA: *Methicillin Resistant S. aureus*; SH: *Shigella*; CA: *C. albicans*

In many instance, the *C. pareira* root concoction is orally taken. It is therefore important to investigate the cytotoxicity levels of this plant to determine its safety. Ethyl acetate and dichloromethane fraction were cytotoxic having CC_{50} of 5.22 and 55.0 µg/ml respectively. However, Petro ether fraction and methanolic fractions were not toxic with CC_{50} > 90.0 µg/ml, the set threshold for toxicity (table 4) (26). Cytotoxicity is like a double edged sword. In one way, it's bad for a drug as it will be lethal to subjects. However, this can be resolved by structural modification with view of improving on their safety. On the other hand, this can be a good news since the cytotoxic fractions can be tapped as candidates for anticancerous drugs [22].

Table 4: Cytotoxicity results of *C. pareira* in µg/ml

Fractions	**CC50 (µg /ml)**
Petro ether	92.47
Dichloromethane	55.0
Ethyl acetate	5.22
Methanol	>500

CC_{50}: Concentration that kills 50 % of the cells of Vero cells.

The observed activity and cytotoxicity could be attributed to the array of phytochemicals that tested positive in the sample extract fractions. These included alkaloids, flavonoids, phenols, terpenoids and anthraquinones. Bioactive molecules are usually found accumulated as secondary metabolites in various parts of the plant and at different concentrations [36]. In this regard, the root is one of the major depository sites of such compounds making it a chief part for herbal bioprospecting. Flavonoids which tested positive in all samples except in petro ether fraction have general antibacterial activity. They have been shown to work by complexing and altering the conformation(s) of microbial proteins thus inactivating microbial enzymes and interfering with bacterial cell wall adhesins. In a similar fashion as terpenoids (also present in all samples except petro ether fraction), they also mediate their antibacterial activity through microbial membrane disruption [36]. Other studies have associated flavonoids with antituberculous activity and it is believed that their mode of action is by inhibiting various pathways in *Mycobacteria* including *de novo* biosynthesis of fatty acid, inhibiting mycolic acid biosynthesis, proteosome 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 [15, 37]. In

addition, recent studies done by [38] indicated that phenolic compounds have antimycobacterial properties although their mode of action is not well known. Similarly, studies have established that alkaloids have both antibacterial and antifungal activities, especially against *S. aureus* and *C. albicans* [39]. The results of this study not only provide the scientific rationalization of the therapeutic uses of *C. pareira* plant in traditional medicine, but also confirms the impact of ethnopharmacological approach when investigating plants for their antimicrobial activity [32].

Table 5: Phytochemical results of fractions

Extract Fraction	V-Ts	A-F	MK-A	D-A	F-P
Petro ether	-	-	+++	++	-
Dichloromethane	+++	++	+++	++	+
Ethyl acetate	+	+	+	-	-
Methanol	++	+++	++	+	+++

V-T, Vanillin test for terpenoids; A-F**,** Ammonia test for Flavonoids; MK-A, Methanolic Potassium hydroxide test for Anthraquenones; D-A, Dragendorff test for Alkaloids; F-P, Ferric Chloride test for Phenols; **-,** Absent phytochemicals**; +,** Low concentration of phytochemicals; **++,** Medium concentration; **+++,** High concentration of phytochemicals.

There is difference in regard to activity and phytochemical profiles in tested fraction in this study and previous work by other scientists. However this difference can be associated with the diversity of plants bioactive compounds which is influenced by genetic characteristics, environmental factors such as climate, altitude and soil type; the period in plants life history when collection took place, the treatment after collection and existence of a distinct phenotype of a particular species (also known as chemical races) [22].

4. Conclusion
Taken together, our findings demonstrate that the *C. pareira* has very high selective potential as a source of novel lead for antituberculous, antibacterial and antifungal drugs. Of particular excitement is its high activity against MTB, *S. aureus*, MRSA, and *C. albicans* which are currently posing great public health challenge due to drug resistance development and as major sources of community and hospital based infections. Noteworthy is methanolic fraction which not only yielded the highest activity with the lowest MIC, but that was also not toxic. We therefore suggest that methanolic fraction provide a viable candidate to be tapped for novel safe antimicrobial drugs.

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6. Competing Interest
The authors declares that they have no competing interests.

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