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Evaluation of Antibacterial Activities and Cytotoxicity of Three Medicinal Plants Used for the Management of *Mycobacterium tuberculosis* and *Staphylococcus aureus* Infections in the North-West Region of Cameroon

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Abstract

Introduction: Chemotherapy is used to combat tuberculosis, and other microbial infections. Unfortunately, resistance has been reported to a vast majority of currently use drugs. The objective of this study was to search for new therapies from plant products for the sustainable management of tuberculosis and *S. aureus* infections. **Materials and Methods:** Crude extracts were obtained by sequential maceration of dry powdered plant material in three solvents; hexane, dichloromethane and methanol. Phytochemical screening to identify active constituents in the crude extracts was done by conventional methods. The antimycobacterial and antimicrobial activity on *M. tuberculosis* control strain H37Ra and a clinical isolate of *S. aureus* respectively, was evaluated at eight different concentrations using the microplatealamar blue assay. Cytotoxicity of the active extracts was evaluated on monkey kidney epithelial cells and assessed using the MTT/formazan assay. **Results:** Twelve crude extracts were obtained, the hexane extract of *Sanseviera liberica* rhizomes (P3rH) showed antimycobacterial activity with a minimum inhibitory concentration (MIC) of 1 mg/mL. The hexane and methanol extracts of *Emilia coccinea*, P2H and P2M respectively, showed antimicrobial activity with MICs of 500 µg/mL and 1000 µg/mL respectively. P2M and P2H had selectivity indices of respectively, 0.1046 and 0.2336. **Conclusion:** This study validates the use of *S. liberica* and *E. coccinea* for the traditional management of tuberculosis and *S. aureus* infections, respectively. Furthermore, it provides a

base for the purification of the active extracts and generation of leads in the search of alternative drugs for the management of these microbial infections.

Keywords

Antibacterial, Cytotoxicity, Medicinal Plants, *Mycobacterium tuberculosis*, *Staphylococcus aureus*

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* is one of the most serious public health problems of the 21st century [1]. Death due to TB is more common when compared to death caused by other bacterial diseases, and this disease is endemic in every country of the world [2] [3]. In 2013, there were 9 million new cases, and 1.5 million deaths caused by TB [4] with approximately, 5.5 million of the cases occurring in Asia, 1.5 million in Africa, 745,000 in the Middle East, and 600,000 in Latin America [5]. Moreover, up to 50 million people are infected with drug-resistant forms of TB with about 500,000 cases of MDR-TB (multidrug-resistant TB) a year worldwide [6] rendering treatment difficult.

Staphylococcus aureus has over the years emerged as a universal and versatile dangerous pathogen in humans [7] and is one of the organisms most encountered in hospital and community acquired infections [8]. Several studies have reported resistance of *S. aureus* to different antibiotics in current use, including penicillin, ampicillin, methicillin, and amikacin [8] [9] [10] thus necessitating the development of new drugs.

Long before the advent of modern medicine, plants were used to treat various diseases and these plants are still being continually used as the basis for the development of many drugs and pharmaceutical products. Cameroon has a rich biodiversity, with ~8620 plant species [11], some of which are commonly used in the treatment of several microbial infections [12]. *Momordica foetida*, *Emilia coccinea* and *Sansevieria liberica* are some of the plants used locally in the management of tuberculosis and *S. aureus* related infections. *Mormodicafoetida* is a member of the plant family *Cucurbitaceae*, widely distributed in tropical Africa and it is a highly valued medicinal plant in this part of Africa [13].

M. foetida is a perennial, herbaceous, climbing plant that produces annual stems of up to 4.5 m long from a rootstock [14]. *Momordica foetida* is used in traditional medicine to treat many ailments like malaria, hypertension, diabetes mellitus, fever, peptic ulcers and as a purgative across many communities in Africa. It is also used as antidotes for venomous stings such as bee stings and also as an analgesic. The warm leaf infusion is drunk to treat anorexia, measles, cough and diarrhea [13]. Tabuti *et al*. [15] carried out an ethnobotanical survey in Uganda to identify some plant species used by traditional medicine practi-

tioners (TMPs) to treat tuberculosis, and *Momordica foetida* was one of the most mentioned plant species used for the treatment of tuberculosis by the TMPs. *M. foetida* was reported to be used traditionally in central Ethiopia in the treatment of swelling wounds [16] and for the treatment of stomach ache in northern Ethiopia [17].

Emilia coccinea belongs to the family *Asteraceae*. This family of plants are mostly woody herbs or shrubs, a few trees and climbing herbs [18]. Commonly called tassel flower, *Emilia coccinea* is an annual plant which grows erect (up to 100 cm) with little or no branches [19]. It is used in traditional medicine for the treatment of several ailments including cough, tumor, rheumatism, fever, dysentery, and it has been reportedly used in the treatment of eye diseases [20]. The root is also used in the treatment of diarrhea [19]. El-Ghani [21] reported the traditional use of *Emilia coccinea* to treat ulcers, hernia and measles in Nigeria. *Emilia coccinea* was also reported to be used in the treatment of gastritis and earache [22].

Sansevieria liberica is a perennial plant that has evergreen leaves and grows up to a height of about 15 - 30 centimeters and is widely distributed in tropical Africa, it belongs to the *Agavaceae* family. This plant has long rhizomes with long fibrous roots and a rapid rate of growth. The leaves and rhizomes have been reported to be used in folkloric medicine for the treatment of asthma, abdominal pains, colic, diarrhea, eczema, gonorrhea, hemorrhoids, hypertension, monorrhagia, piles, sexual weakness, snake bites and wounds of the foot [23]. Ajibesin [24] carried out an ethnobotanical in AkwaIbom state, Nigeria, and reported the use of *Sansevieria liberica* traditionally for treatment of eczema and snake bites.

This study evaluated the antibacterial activity of these three plants as a base for the development of new therapies in the sustainable management of *M. tuberculosis* and *S. aureus* infections.

2. Materials and Methods

2.1. Study Site

This study was carried out from June 2018 to October 2019. The plants were collected in Bambili, Tubah subdivision Northwest region, Cameroon (**Figure 1**). The extraction was done at the natural products laboratory of the Chemistry department of the University of Buea. Antibacterial screening was carried out at the tuberculosis unit of the Buea regional hospital, Southwest Region, Cameroon. Cytotoxicity screening was carried out at the ANDI Centre of Excellence for Onchocerciasis Drug Research, Biotechnology unit, Faculty of Science, University of Buea.

2.2. Plant Collection and Identification

Two traditional healers and six indigenes of Bambili, in the North-West Region of Cameroon were interviewed on the plants commonly used in the local treatment of bacterial-related ailments. Three medicinal plants namely: *Momordica*

foetida, *Emilia coccinea* and *Sansevieria liberica* were harvested. These plants were identified, and voucher specimens deposited at the National Herbarium in Yaoundé, Cameroon with the following voucher numbers assigned: 33420/HNC (*Momordica foetida*), 59184/HNC (*Emilia coccinea*), and 43510/HNC (*Sansevieria liberica*).

2.3. Preparation of Crude Extracts

This was done according to the method used by Samje *et al*. [26] with some slight modifications.

The leaves and rhizomes of the three plants were air-dried and later ground to fine powder. The powder for each plant part was then weighed and for each 200 g of powder material, 1 L of solvent was used for maceration. The ground materials were then sequentially submerged and macerated for 72 hours in three solvents successively starting with hexane then dichloromethane and finally methanol. After 72 hours of maceration with hexane, the mixture was filtered and the filtrate concentrated to obtain the hexane fraction of the crude extract using a rotary evaporator (BUCHI Rotavapor R-200, Switzerland). The procedure was repeated sequentially with the debris with dichloromethane and methanol [26]. Maceration was repeated once for each solvent. The residual solvent was removed by air drying at room temperature for 14 days after which the obtained crude extract was then stored at −20˚C.

2.4. Preparation of Crude Extract Stocks

Crude extract stock solution of 25 mg/mL was prepared by weighing 25 mg of extract on a sensitive scale and dissolving in 1 ml of dimethyl sulfoxide (DMSO solvent grade >99.8%, from Sigma-Aldrich, Germany). The stock solutions were then stored at 20˚C till when needed for biological assays.

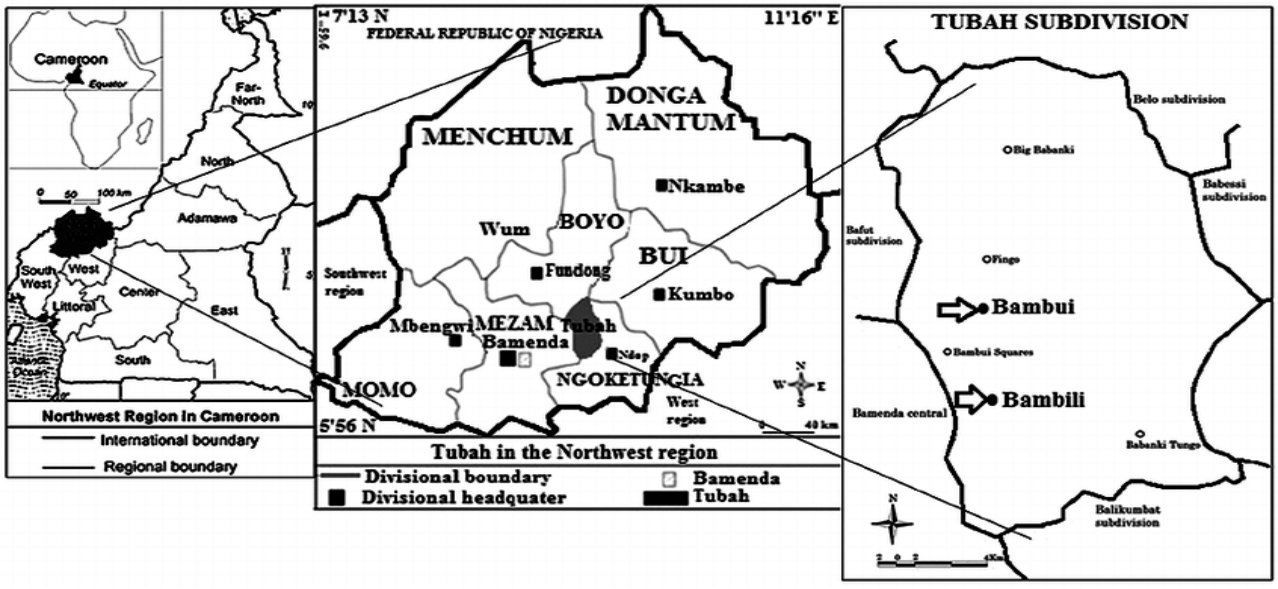

Figure 1. Plant collection area (Source: Zéphyrin *et al*., [25]).

2.5. Culture of *M. tuberculosis* Control Strain, H37Ra

M. tuberculosis was cultured in Middlebrook 7H9 medium supplemented with MGIT (Mycobacteria Growth Indicator Tube) and the antibiotic, MGIT PANTA. Under sterile condition, five milliliters (5 mL) of Middlebrook 7H9 supplemented medium was transferred into a 50 mL sterile tube followed by 0.5 mL of freezer stock of *Mycobacterium tuberculosis* control strain H37Ra (25177 ATCC) [27]. The resulting solution was vortexed and incubated at 37˚C (HeraeusHERAcell 150, Germany) for 14 days. After 14 days of culture, the optical density (OD) was read at 650 nm using a universal microplate reader (BioTek, EL_X800). The bacteria culture was further diluted using Middlebrook 7H9 supplemented medium to obtain a final OD reading at 650 nm between 0.2 - 0.3. A 1/1000 dilution was done and this was then used to set up the assay plates [28].

2.6. Culture of Clinical Isolate of *Staphylococcus aureus*

Clinical isolate of *S. aureus*, isolated from the urine of a five-year-old boy, was obtained from the Buea Regional Hospital laboratory. To prepare a 40 mL slant medium for *S. aureus*, 4.44 g of mannitol salt agar was weighed using a sensitive electronic scale balance and dissolved in sterile distilled water. The solution was autoclaved using a pressure pot at 121˚C for 15 minutes. It was then placed in a such a way that the medium will slant upon cooling. After solidification of the medium, *Staphylococcus aureus* colonies were inoculated from the refrigerated plate culture in a sterile environment into the mannitol salt agar slant and incubated at 37˚C for 18 - 24 hours. After 24 hours of incubation of *S. aureus*, colonies were picked and inoculated into sterile nutrient broth and the solution was adjusted to match a McFarland standard 1, after which a 1:20 dilution was done. This was then used to set up the assay plates.

2.7. Phytochemical Screening of Bioactive Compounds

Phytochemical properties of the active crude extracts such as alkaloids, flavanoids, steroids, saponins, cardiac glycosides, phenolics and tannins were determined using standard methods as described elsewhere [29] [30].

2.8. Antibacterial Activity Testing

The Microplate Alamar Blue Assay (MABA) used by Nkenfou *et al*. [27] was used with some slight modifications made, to test the anti-mycobacterial and antimicrobial activities of the crude extracts. This method operates as a simple colorimetric mode with visual reading, blue to pink change indicates viability of the bacteria whereas a blue coloration indicates an anti-mycobacterial or antimicrobial effect of the crude extract on the bacteria. Absorbance was obtained using a microplate reader (BioTek, EL_X800) at 540 nm and 630 nm.

2.8.1. Primary Screening for Antimycobacterial Activity

This was done in order to eliminate inactive extracts. An extract concentration of 1 mg/mL was used as the peak or starting concentration. We placed 100 µL

extract in 96 wells plate followed by 100 µL of the *M. tuberculosis* control strain to obtain a final volume of 200 µL. Isoniazid was dissolved in DMSO and used as a positive control at a concentration of 1.6 µg/mL, while negative control wells contained only the diluent (DMSO). The plate was then sealed and placed in an incubator (HeraeusHERAcell 150, Germany) at 37˚C. Experiments were done in triplicates. After seven days of incubation, 30 µL of alamar blue was added into each well and incubated overnight. Wells that remained blue after overnight incubation in the presence of alamar blue indicated activity of the corresponding crude extract while those that turned pink indicated inactivity of the corresponding extracts on *M. tuberculosis*.

2.8.2. Primary Screening for Antimicrobial Activity

An extract concentration of 1000 µg/mL was prepared, and this served as the starting concentration. One hundred microliter of the *S. aureus* which had been adjusted to a McFarland standard 1 and further diluted in a 1:20 proportion was then added into the wells containing 100 µl of crude extracts in the 96-well plate. Streptomycin sulphate at 0.2 mg/mL was used as a positive control while the negative control wells contained only the diluent. Experiments were done in triplicate and repeated once. The plate was then sealed and placed in an incubator (HeraeusHERAcell 150, Germany) at 37˚C. After 12 hours of incubation, 30 µL of alamar blue was added into each well and incubated for 8 - 12 hours. Wells that remained blue after incubation, in the presence of alamar blue indicated activity of the corresponding crude extract and wells that turned pink indicated inactivity of the corresponding crude extracts on *S. aureus.*

2.8.3. Secondary Screening of Extracts

Extracts that showed activity during the primary screening were further screened at 8 different concentrations starting at 1000 µg/mL. For *M. tuberculosis* assay, 100 µL of supplemented Middlebrook 7H9 broth was used while 100 µL of nutrient broth was used for *S. aureus.* One hundred microliters of the corresponding bacteria were then introduced into the wells, the plate was sealed and incubated at 37˚C for 12 hours for *S. aureus* and seven days for *M. tuberculosis* after which 30 µL of alamar blue was added and further incubated for another 12 hours for *S. aureus* and for about 12 - 18 hours for *M. tuberculosis*.

2.8.4. Determination of MIC and Percentage Inhibition

The MIC was determined as the lowest concentration which did not cause a change in color from blue to pink and inhibited at least 90% of bacteria from growing. The percentage of inhibition of growth of bacteria was calculated using Equation (1) [31].

Equation (1) is:

$$\frac{\left[\left(\varepsilon_{ox}\right)\lambda_2 A\lambda_1 - \left(\varepsilon_{ox}\right)\lambda_1 A\lambda_2 \text{ of test agent dilution}\right]}{\left[\left(\varepsilon_{ox}\right)\lambda_2 A^0\lambda_1 - \left(\varepsilon_{ox}\right)\lambda_1 A^0\lambda_2 \text{ of untreated positive growth control}\right]} \times 100 \quad (1)$$

where;

$\lambda_1 = 540$, $\lambda_2 = 630$;

$(\varepsilon_{ox})\lambda_1$ = molar extinction coefficient of alamarBlue oxidized form (blue) at 540 nm;

$(\varepsilon_{ox})\lambda_2$ = molar extinction coefficient of alamarBlue oxidized form (blue) at 630 nm;

Aλ_1 = Observed absorbance reading for test well at 540 nm;

Aλ_2 = Observed absorbance reading for test well at 630 nm;

$A^0\lambda_1$ = Observed absorbance reading for positive control well at 540 nm;

$A^0\lambda_2$ = Observed absorbance reading for positive control well at 630 nm.

2.9. Cytotoxicity Activity

Preparation of Monkey Kidney Cells for Cytotoxicity Assays

This was done according to the method described by Samje *et al*., [25] with some modifications.

Monkey kidney epithelial cells (LLC-MK2) purchased from the American Type Culture Collection (ATCC, Virginia, USA) were proliferated in complete culture media (CCM). CCM is made up of RPMI-1640 (incomplete culture medium, ICM) supplemented with 25 mM HEPES, 2 g/L sodium bicarbonate, 0.3 g γ-irradiated L-glutamine powder, 5% newborn calf serum, 200 units/mL penicillin, 200 µg/mL streptomycin and 0.25 µg/mL amphotericin B (Sigma, USA); pH 7.4. Using a water bath at 37˚C, frozen cells were thawed by agitating gently. As soon as the content of the vial was thawed, the vial was removed from the water bath and 70% ethanol was sprayed on it for decontamination. The cell suspension was centrifuged at 125× g for 10 minutes (Eppendorf centrifuge 5810R, Germany). The supernatant was discarded and the cell pellet re-suspended in fresh CCM. The cells were then distributed in 75 cm^3 falcontissue culture flasks and cultured at 37˚C under an atmosphere of 5% CO_2 in humidified air (Heracell 150i CO_2 incubator, USA) until they were ready to be sub cultured. Once the cells became fully confluent, the old media was decanted and the cells were dislodged with 1X trypsin-EDTA (SIGMA life science) in ICM. The flask was allowed to stand for 15 minutes in a CO_2 incubator while occasionally observing using an inverted microscope (Nikon Eclipse TS100, China) to ensure that the cells were completely dislodge from the bottom of the flask. Using an appropriate volume of ICM, the dislodged cells were re-suspended in a 50 mL falcon centrifuge tube and centrifuged at 125 ×g for 10 minutes. After centrifugation the supernatant was discarded and the cell pellet re-suspended in 3 mL of CCM. Appropriate volumes of CCM corresponding to a dilution that will give 5000 cells/100µl were added. The cell suspension was then seeded into 96-well flat bottom microtitre plates and incubated for three days using a CO_2 incubator for the cells to grow and become fully confluent.

Cytotoxicity assay was done according to the method described by Cho-Ngwa *et al*. [32]. After the monkey kidney epithelial cells became fully confluent in the 96 well plates, the medium in which the cells proliferated was flushed and the plate blotted. After that 150 µL fresh CCM was introduced into the wells. The

active extracts were prepared 4× concentrated in a total volume of 300 µL in the first rows of a 48-well plate and a two-fold serial dilution was done down the plate and the residual 150 µL left in the pipette was discarded. To the LLC-MK2 cells in 150 µL of fresh CCM in the 96-well plate, 50 µL of the different dilutions of the extracts was added to obtain a final volume of 200 µL. A positive control was done using auranofin and a negative control was done using DMSO. Experiments were done in duplicate. The cells were then incubated at 37˚C and 5% CO_2 for five days (Heracell 150i CO_2 incubator, USA). An examination of the deformities and degree of detachment was done daily using an inverted microscope (Nikon Eclipse TS100, China). After five days of incubation, the supernatant was removed by gently inverting, flicking and blotting the plate. The wells containing colored extracts were rinsed twice with 100 µL of ICM per well. A 1 mg/ml MTT (Sigma, life science) solution was prepared using ICM as the solvent, and 100 µL of this solution was added to the cells in each well and incubated for four hours. After which the untransformed MTT was removed by gently inverting, flicking and blotting the plate. DMSO was then added (100 µL) in each well and the plate was shaken vigorously on a shaker in order to solubilize the blue formazan formed. Optical densities were then read using an automatic microplate reader (SoftMax, Molecular Devices, USA) at a wavelength of 595 nm.

The percentage inhibition of formazan formation was calculated using the formula;

Equation (2) is;

$$\text{Percent inhibition}(\%) = \frac{[\text{OD of control} - \text{OD of treatment}]}{\text{OD of control}} \times 100 \quad (2)$$

The CC_{50} was determined using GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). The CC_{50} is the concentration at which cellular effects are inhibited by 50%.

Equation (3) is;

$$\text{SI} = \frac{CC_{50} \text{ of extract on mammalian cell}}{\text{MIC of extract on M. tuberculosis}} \quad [33] \quad (3)$$

3. Results

3.1. Antimycobacterial Activity of Crude Extracts

A total of 12 crude extracts were screened at a single highest concentration of 1 mg/mL. The hexane extract of the rhizomes of *Sansevieria liberica* (P3rH) showed activity against *Mycobacterium tuberculosis* at 1 mg/ml. In the secondary screen, P3rH recorded an MIC of 1 mg/ml with a percentage inhibition of 94.81%. **Figure 2** shows the linear relationship between the concentration of P3rH and percentage inhibition on *M. tuberculosis.*

3.2. Antimicrobial Activity of Extracts

The hexane and methanol extracts of *Emilia coccinea*, P2H and P2M respectively,

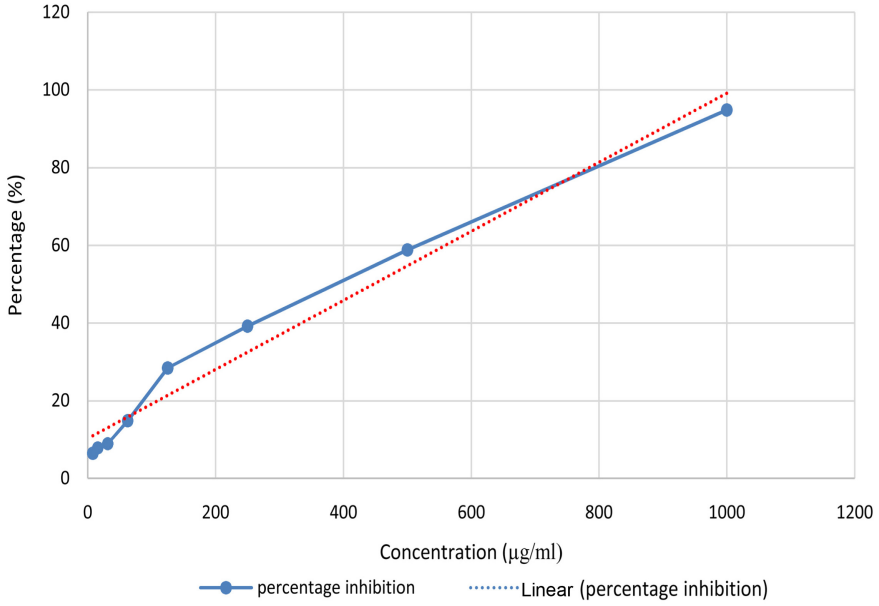

Figure 2. Linear relationship between concentration of P3rH and percentage inhibition on *M. tuberculosis*.

showed activity against *S. aureus* at a maximum concentration of 1 mg/ml in the primary screen. In the secondary screen, P2H had an MIC of 0.5 mg/mL and percentage inhibition of 90.72% while P2M had an MIC of 1 mg/mL and a percentage inhibition of 93.76%. **Figure 3** and **Figure 4** show the linear relationship between the concentration of P2H and P2M respectively and the percentage inhibition on *S. aureus*.

3.3. Cytotoxicity Studies

The cytotoxic concentration, minimum inhibitory concentration and corresponding selectivity index values for the different crude extracts were determined and P2H was seen to have the highest selectivity index (0.234) and lowest MIC (0.5 mg/mL) as shown in **Figure 5**.

3.4. Phytochemical Identification of Extracts

A total of seven phytochemicals namely, steroids, alkaloids, cardiac glycosides, phenolics, tannins, flavonoids and saponins were identified in the different extracts. Flavonoids and cardiac glycosides were seen to be present in all the extracts while alkaloids were present in just two of the extracts: the dichloromethane extract of *Emilia coccinea* (P2D) and the dichloromethane extract of the leaves of *Sansevieria liberica* (P3lD). Phenolics were seen to be present only in the methanol extract of the rhizomes of *Sansevieria liberica* (P3rM). Out of the seven phytochemicals screened, P3lD the highest number of phytochemicals (six) while the hexane extract of *Momordica foetida* (P1H), the hexane extract of *Emilia coccinea*, and the dichloromethane extract of the roots of *Sansevieria liberica* (P3rD) showed the presence of four phytochemicals (**Table 1**).

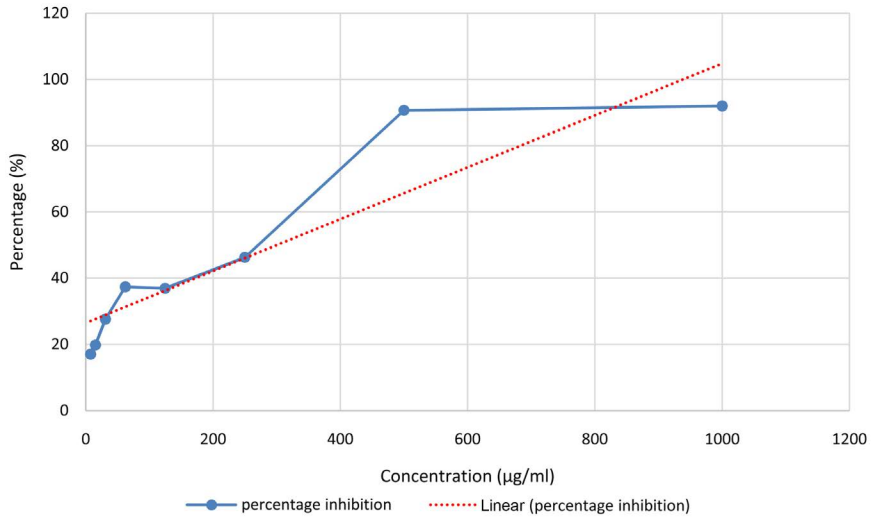

Figure 3. Linear relationship between concentration of P2H and percentage inhibition on *S. aureus*.

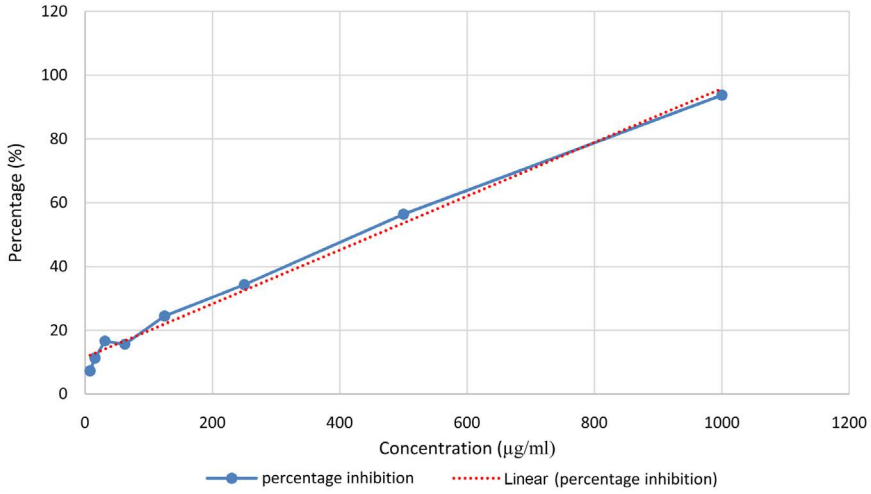

Figure 4. Linear relationship between concentration of P2M and percentage inhibition on *S. aureus*.

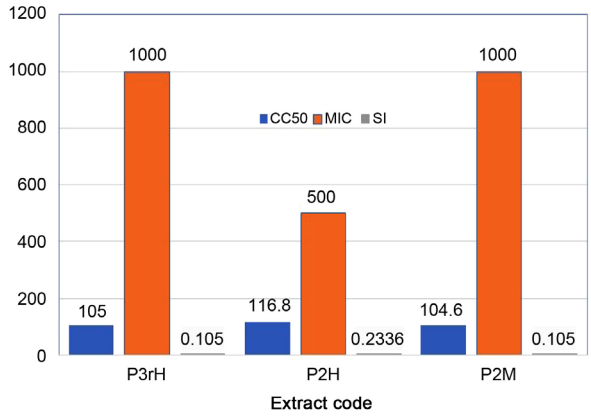

Figure 5. Representation of CC50, MIC and SI (%) values of active extracts.

Table 1. Phytochemicals screened in crude extracts.

Extracts	Phytochemical Constituents						
	Steroids	Alkaloids	Cardiac glycosides	Phenolics	Tannins	Flavonoids	Saponins
P1H	+++	−	++	−	−	+	++
P2H	+++	−	+++	−	−	+	+
P3lH	++	−	++	−	+++	+	++
P3rH	+++	−	+	−	++	+	+
P1D	+++	−	+++	−	++	+	+
P2D	++	+	+++	−	++	+	−
P3lD	++	+	++	−	+	+	++
P3rD	++	−	++	−	−	+	++
P1M	++	−	+++	−	+	+	+
P2M	+++	−	+++	−	+	+	+
P3lM	++	−	++	−	+	+	+++
P3rM	−	−	+	+	+	+	++

Key: + = low concentration, ++ = moderate concentration, +++ = high concentration, − = negative or absent. P1H = hexane extract of *Momordica foetida*, P2H = hexane extract of *Emilia coccinea*, P3lH = hexane extract of *Sansevieria liberica* leaves, P3rH = hexane extracts of *Sansevieria liberica* rhizomes, P1D = dichloromethane extract of *Momordica foetida*, P2D = dichlromethane extract of *Emilia coccinea*, P3lD = dichloromethane extract of *Sansevieria liberica* leaves, P3rD = dichloromethane extract of *Sansevieria liberica* rhizomes, P1M= methanol extract of *Momordica foetida*, P2M = methanol extract of *Emilia cocinea*, P3lM = methanol extract of *Sansevieria liberica* leaves, P3rM = methanol extract of *Sansevieria liberica* rhizomes.

4. Discussion

This study aimed at contributing to the search for new therapies for the better management of *M. tuberculosis* and *S. aureus*. We found that the hexane extract of the rhizomes of *Sansevieria liberica* had activity at a concentration of 1 mg/mL and inhibited the growth of *Mycobacterium tuberculosis* by 94.81%. The hexane and methanol extracts of *Emilia coccinea* were found to have activity at 500 µg/mL and 1000 µg/mL and inhibited growth of *S. aureus* by 90.72% and 93.76% respectively.

Phytochemicals identified in this study have a wide range of biological activities. Saponins are widely distributed amongst plants and it is believed that saponins naturally act to protect the plant against pathogens [34]. Their main biological activities include haemolytic, molluscicidal, anti-inflammatory, antimicrobial, anti-parasitic, cytotoxic and anti-tumor [35]. Total phenolics generally possess antimicrobial activities which provide chemical barriers for invading microorganisms [33]. On the other hand, flavonoids inhibit bacterial growth by inhibition of DNA gyrase, cytoplasmic membrane function and energy metabolism [36]. Tannins exhibit antioxidant, antimicrobial and antiviral effects [37]. The plant extracts also contained steroids, which have anti-inflammatory effects [38], and alkaloids that have been reported to exert analgesic, antispasmodic and antibacterial activities [39].

The antimycobacterial and antimicrobial activity of the 3 crude extracts may be related to the presence of steroids, tannins, saponins and flavonoids which have been reported to have antibacterial activities. All 3 active extracts had a high concentration of steroids which may justify their antibacterial activities. The results of this study are consistent with that of the study carried out by Ndam *et al*., [40] who carried out the phytochemical screening of 20 plants including two of the plants (*Emilia coccinea* (P2) and *Momordica foetida* (P1)) whose bioactive compounds were identified in this study. Phytochemical screening of *Sansevieria liberica* (P3), showed the presence of flavonoids, saponins, alkaloids and tannins which corroborates with the results obtained by Ikewuchi *et al*. [41]. These results however differ from that reported by Adelanwa and Habibu [42] who reported the absence of saponins and alkaloids but reported the presence of cardiac glycosides in *S. liberica* which were reported to be present in this study.

Many studies have been done on *Sansevieria liberica* (P3), but to the best of our knowledge, this is the first study that evaluated the antimycobacterial activity of *Sansevieria liberica*. The antidiarrheal effect of this plant was evaluated and reported by Adeyemi *et al.* [43] even though this study showed no activity of *S. liberica* on *S. aureus*. Adelanwa and Habibu [42] reported that *Sansevieria liberica* has an antimicrobial activity against *S. aureus* and *B. cereus* which is consistent with our findings on the activity of the rhizomes extract on *M. tuberculosis*. The antimycobacterial activity of *S. liberica* may be related to the presence of saponins which protects the plant against invading pathogens and has been reported to have antimicrobial and antioxidant properties [35], and also due to the presence of tannins which have also been reported to have antimicrobial activities [37]. The low activity of the rhizome extract may have been due to presence of the complex cell wall of *Mycobacterium tuberculosis* that is characterized by the presence of mycolic acids and its ability to resist dryness.

So many studies have been carried out on the antimicrobial activity of *Emilia coccinea* (P2). Erhabor *et al.* [44] carried out a study in Nigeria where they studied the antimicrobial activity of the aqueous and methanol extracts of *Emilia coccinea* on a range of bacteria including *S. aureus* and they found out that both extracts had activity on *S. aureus* with the bacteria being more sensitive to the methanol extract with an inhibition diameter of 20.33 ± 2.73 mm. These results are in line with our findings in this study which indicated activity of the methanol extract of *Emilia coccinea* on *S. aureus*. In another study carried out by Mihigo *et al*. [45] where they studied the preliminary GC-MS profiling and anti-bacterial activity investigation of *Emilia coccinea*, and found out that the hexane extract of the plant was most active against *S. aureus* and *E. coli*, with an MIC of 500 - 1000 μg/mL, while the dichloromethane extract was found to be inactive. These results support the results obtained for this study which showed similar MIC of hexane extract of the plant against *S. aureus. S. aureus* has been reported to be one of the major organisms that cause diarrhea and stomach dis-

orders in humans [46] [47]. In contrast to the results obtained from this study, Okiei *et al*. [20] reported inactivity of the hexane extract of *Emilia coccinea* on a range of bacteria including *S. aureus*. The results obtained from this study could thus be used as a scientific support for the use of the plant for the management of stomach related disorders like diarrhea. *S. aureus* has also been isolated from wounds [48], and the activity of *Emilia coccinea* on this bacterium as shown in this study may constitute a scientific support for the traditional use of this plant in the treatment and management of wounds.

The most cytotoxic extract was P2M which had the lowest SI value. P2H was the least cytotoxic with an SI value of 0.2336. To the best of our knowledge, this is the first study that evaluated the cytotoxicity activity of *Sansevieria liberica* on normal or non-cancer cell lines. Cytotoxicity activity on *Sansevieriatrifasciata*, a plant of the same family as *Sansevierialiberica* has been reported by others. Berame *et al*. [49] carried out a study on preliminary phytochemical screening and toxicity test of leaf and root part of snake plant (*Sansevieria trifasciata*) and they found out that the root part (rhizome) was more toxic as compared to the leaves. These results corroborate that obtained from this study which indicates cytotoxicity of the rhizomes of *Sansevieria liberica.* From the results obtained in this study, P2M which is the methanol extract of *Emilia coccinea* was found to be the most cytotoxic to the LLK-MK2 cells which is similar with the results obtained by Shylesh and Padikkala [50] in a study they carried out on *Emilia sonchifolia* which is a member of the same family and genus as *Emilia coccinea.* They found out that the methanol extract of *Emilia sonchifolia* was cytotoxic to daltons lymphoma (DL), Ehrlich ascites carcinoma (EAC) and mouse lung fibroblast (L-929) cells.

5. Conclusions

This study aimed at contributing to the search for new therapies for the better management of *M. tuberculosis* and *S. aureus*, and out of the three plants that were screened, *Sansevieria liberica* showed activity against *Mycobacterium tuberculosis* and *Emilia coccinea* had activity against *S. aureus*. *Momordica foetida* showed no activity on any of the bacteria. This study supports the acclaimed activities of *S. liberica* and *E. coccinea* in the traditional management of respectively tuberculosis and *S. aureus* infections. It equally unveils new sources of potential anti-bacterial drug candidates that can be exploited in the development of novel antimycobacterial and antimicrobial drugs.

This study was limited to screening of gram positive bacteria. Screening the extracts on gram negative bacteria and a range of gram positive bacteria would give more insight into the antibacterial activity of these plants.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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