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Antimycobacterial activities, cytotoxicity and phytochemical screening of extracts for three medicinal plants growing in Kenya

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Tuberculosis (TB), an airborne disease, is among the ten leading deadly diseases worldwide. Despite the efforts of WHO and its partners to eradicate it, it is still a public health issue especially with the rise of multi-drug resistant tuberculosis (MDR-TB) and extensively drug- resistant tuberculosis (XDR-TB). *Commiphora* species (Burseraceae family) are known in the Kenyan traditional medicine to treat respiratory diseases including TB. In the search of new anti-TB alternative drugs, plant materials from *Commiphora mildbraedii* Engl. (root bark and stem bark), *Commiphora edulis* (Klotzsch) Engl. (stem bark and leaves) and *C. ellenbeckii* Engl. (Stem bark and leaves) were tested for antimycobacterial activity, cytotoxicity and phytochemistry. 100 g of the powdered plant materials were macerated using the serial method with solvents of increasing polarity. Aqueous extraction was carried out by decoction. The microbroth dilution method was used to determine the antimycobacterial activity (MIC) against a model *Mycobacterium smegmatis* ATCC607 while the cytotoxicity evaluation (CC_{50}) was carried out using the MTT assay. The most active extract was fractionated using preparative TLC and fractions were analysed by GC-MS. Thirty extracts were obtained from the 6 different plant materials and eleven of them exhibited the antimycobacterial activity with the methanolic extracts of the stem and root bark of *C. mildbraedii*, and the aqueous extract of the *C. ellenbeckii* leaves exhibiting high activities (MIC= 0.39, 0.78 and 0.78 mg/L respectively). The MTT assay showed no or low cytotoxicity. The GC-MS analysis of the preparative TLC fractions from the methanolic extract of *C. mildbraedii* revealed the presence of 42 compounds belonging to 10 different classes of phytochemicals. Lup-20(29)-en-3-one and o-xylene were the most abundant. Except o-xylene and α-terpineol, all the compounds were detected for the first time in the *Commiphora* genus. These findings justify the ethnomedicinal uses of *Commiphora* species in TB treatment.

Key words: *C. mildbraedii*, *C. ellenbeckii*, *C. edulis*, antimycobacterial activity.

INTRODUCTION

Tuberculosis (TB) is an airborne contagious disease caused by *Mycobacterium tuberculosis*. Despite all the efforts of the World Health Organization (WHO) and its partners to eradicate this disease, TB continues to be a public health concern. TB is one of the top 10 causes of death worldwide. In 2017, there were an estimated 10 million new TB infections worldwide. People co-infected with HIV and TB accounted for 9% of the total

and the majorities were adults up to 90% (aged ≥15 years). TB is also the main cause of deaths related to antimicrobial resistance and the leading killer of people with HIV. Globally, the TB mortality rate has widely decreased from 23% in 2000 to 16% in 2017 and the TB incidence rate is generally reducing to about 2% yearly worldwide (WHO, 2018). This reduction of the incidence is contrasted with the continuous rise of drug resisting TB strains, hence the need for developing new intervention methods. In 2017, an estimation of 558 000 new cases with resistance to rifampicin (RR-TB) was reported and eighty-two percent (82%) of those cases had multi-drug resistance-tuberculosis (MDR-TB). Moreover, an estimated 8.5% of people with MDR-TB had extensively drug-resistant TB (XDR- TB). About 23% of the world's population has latent TB infection and is therefore prone to develop active TB disease during their lifetime (WHO, 2018). It is for these major reasons that researches carried out these days, focus more on developing new alternatives to counteract this hectic problem of drug resistance in bacterial infections and particularly in TB.

Synthetic drugs are not the only focal point, nature provides also a great reservoir of medicinally active ingredients that could alleviate or suppress this phenomenon of drug resistance (Zumla et al., 2013). *Commiphora* plants (myrrh plants) are well known in various cultures to have medicinal virtues including treating infectious diseases. The myrrh plants (*Commiphora* sp.) belonging to the Burseraceae family are among the most popular plants that have been in use since time immemorial.

The myrrh genus (Burseraceae family) is the richest in terms of species among the flowering plants of the Burseraceae family. The plant list reports that it comprises approximately 208 species (https://www.plantlist.com). They are shrubs and trees mostly distributed throughout the sub-tropical regions of Africa, the western Indian Ocean islands, the Arabian Peninsula, India and Vietnam (Daly et al*.,* 2011). The genus *Commiphora* has been exploited worldwide as a natural drug to treat pain, skin infections, inflammatory conditions, diarrhea, periodontal diseases, and wounds (Nomicos, 2007; Abdul-Ghani et al*.*, 2009). Its ethnomedicinal uses, pharmacology and phytochemistry have been thoroughly reviewed by Hanuš et al. (2005) and Shen et al. (2012).

Egyptians used *C. myrrha* early 4000 years ago, in the process of embalming bodies and its perfume was used in spiritual rites (Abdul-Ghani et al*.*, 2009). In the Islamic inspired culture, it was utilized for the treatment of intestinal parasites, diarrhea, wound treatment, persistent chest ailments (Ghazanfar, 1994). Chinese medicine exploits *C. myrrha* for the treatment of wounds, dysmenorrhea, abnormal blood clotting (thrombosis) (Hanuš et al*.*, 2005), for toothache, oral ulcer, tumor, inflammatory diseases and for acroanesthesia (Shoemaker et al., 2005). Pharmacological studies carried on *C. myrrha* proved that it exhibits good antimicrobial activity. It was tested against Gram-positive and Gram-negative bacteria and some fungal strains, and it inhibited the growth of the tested strains (Omer et al., 2011, Alhussaini et al*.,* 2015).

South Africans valued *C. molmol* (or *C. mollis*) for the treatment of fever (malaria and typhoid), wound healing, cancer, ulcer, rheumatic conditions, colds, nasal congestion and coughing (Van Wyk et al*.,* 2002). It is reported that the methanolic extracts of *C. molmol* exhibited high antibacterial activity against Gram-positive bacteria than tested Gram-negative bacteria with MIC ranging from 31.25 to 500 µg/mL (Abdallah et al*.,* 2009). In another study, the oil from *C. molmol* has strong activity against clinical *S. aureus* isolates including multi-drug resistant strains (Mohammed and Samy, 2013).

In Nigeria, the seed decoction of *C. Africana* is traditionally used for expelling tapeworms (McGuffin et al*.*, 2000). An alkaloid isolated from *C. africana* presented antimicrobial activities against all the test microorganisms (Banso and Mann, 2006). On the other hand, the chloroform and methanolic extracts (leaves and stem) of ten South African *Commiphora* species including *C. africana*, *C. schimperi*, *C. grandulosa*, *C. marlothii*, *C. mollis*, *C. neglecta*, *C. pyracanthoides*, *C. tenuipetiolata*, *C. viminea* were tested for the antimicrobial activity and were found to possess high bioactivity with MICs of 0.01-8.00, 0.25-8.00 and 1.00-8.00 mg/ml against Gram-positive bacteria, yeasts and Gram-negative bacteria respectively (Paraskeva et al*.,* 2008).

In folk medicine, the bark infusion of *C. edulis* was used to treat malaria and its roots, leaves, and stem are used in the treatment of stomachache, menstrual problems and spirits related illnesses (Orwa et al., 2009). Paraskeva et al*.* (2008) tested *C. edulis* for the antimicrobial activity and found that it was able to inhibit the growth of yeasts and Gram-positive and Gram- negative bacteria tested with MIC ranging from 2-8 mg/mL.

The literature provides a number of reports where some *Commiphora* species were tested for antimycobacterial activity using different *Mycobacteria* model strains and the pathogenic *M. tuberculosis*, a pre-requisite step in TB drug discovery.

The essential oil from the fresh aerial parts of *C.*

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opobalsamum L., a plant used by Saudi Arabians to treat infectious problems (Shen et al., 2007) was evaluated for the antimicrobial activity and exhibited a good activity with IC_{50} of 80, 90, 150 and 15 µg/mL against *Candida glabrata*, *C. krusei*, *Cryptococcus neoformans*, and *Mycobacteria intracellulare* respectively (Al-Massarany et al., 2008). The yellowish sap from the stem of *C. mukul* (guggul) is well known in Ayurvedic medicine over 2000 years for the treatment of arthritis inflammation, hyperlipidemia related diseases and to improve the hepatic antioxidant defense system (Al-Rejaie, 2012). It is also known to treat some infections and is reported to exhibit good antimycobacterial activity against two models of Mycobacteria, *M. aurum* and *M. smegmatis* with a MIC 62.5 µg/mL for both (Newton et al., 2002).

Tanzanian pastoralists use aromatic saps from the stem bark of *C. eminii* in the treatment of skin infections, gastrointestinal infections, wounds and as well as dry and blood cough (Erasto, 2012). Two sterols (4 -methyl-cholest-7-en-3-ol and cholest-7-en-3-ol) isolated from the sap of *C. eminii* stem bark were tested for antimycobacterial activity against *Mycobacterium madagascariense* and *Mycobacterium indicus pranii* and only the second sterol (cholest-7-en-3-ol) exhibited antimycobacterial activity with MIC values of 1.6 mg/mL against both *Mycobacteria* strains used.

Indigenous tribes in the Northeast of Brazil utilize *C. leptophloeos* as an infusion, tea or syrup for the treatment of their illnesses, such as infectious and inflammatory ones (Silva et al., 2011). Hinokinin, a compound that was isolated from *C. leptophloeos* presented antimicrobial activity against a variety of microbes with MIC values ranging from 0.0485 to 3.125 mg/mL (against different *S. aureus* clinical isolates) and showed a bactericidal activity against Methicillin- resistant *Staphylococcus aureus* (MRSA) isolated from blood with of MMC= 0.40 mg/mL. Additionally, the *C. leptophloeos* extracts were tested for antimycobacterial and it exhibited moderate activity against *M. smegmatis* (MIC=12.5 mg/mL) and *M. tuberculosis* (MIC=52 mg/mL) (De Souza et al., 2017). The Maasai community of southern Kajiado District of Kenya utilizes *Commiphora* sp. for various medicinal uses including the treatment of killing chiggers, open wounds, as a skin moisturizer and for the treatment of respiratory diseases such as coughing and tuberculosis (Kiringe, 2006).

Kenyan biodiversity is so rich in *Commiphora* species but less has been subject to studies for proving the above mentioned ethnomedicinal uses. Therefore, this study was designed to establish the antimycobacterial, cytotoxicity and phytochemical properties of selected African *Commiphora* species growing in Kenya. These include *Commiphora mildbraedii* Engl., endemic in regions ranging from Ethiopia to Kenya, and *Commiphora edulis* (Klotzsch) Engl. And *Commiphora ellenbeckii* Engl. found abundantly in Kenya, Ethiopia and Tanzania (Vollesen, 1989). This work intends then to verify the efficacy and safety of the three *Commiphora* plants used in the treatment of TB by the Kenyan local communities; and to screen their bioactive compounds.

MATERIALS AND METHODS

Plant collection

Plant materials consisting of *C. mildbraedii* stem and root bark, *C. edulis* stem bark and leaves and *C. ellenbeckii* stem bark and leaves were identified by a taxonomist and collected in various locations of Kenya. *C. edulis* was collected at Chekbor, Marakwet County and *C. mildbraedii* and *C. ellenbeckii* were collected at Kibwezi forest, Kenya. Voucher specimen were prepared and deposited at the University of Eldoret Herbarium, Eldoret, Kenya where they were assigned voucher numbers: CFW/31/1/19/003 for *C. edulis*, CFW/3/2/19/008 for *C. mildbraedii* and CFW/3/2/19/010 for *C. ellenbeckii*.

Sample processing

Drying of the plant materials was carried out in open-air and well-ventilated room for about one month. Once the plant materials were dry, grinding was undertaken using an electric mill “Christy 8 MILL, No. 51474” to obtain a coarse powder.

Chemical reagents

Chemicals that were utilized in the various experiments were bought from Merck-Sigma Aldrich. All the reagents were of analytical grade.

Plant extracts preparation

Organic solvent extracts were prepared using the serial or successive extraction method with solvents of increasing polarity: n-hexane-dichloromethane (DCM)- ethyl acetate (EtOAc)- methanol (MeOH). 100 g of the powdered samples were soaked successively. For each solvent, the maceration was carried twice before using the following solvent. The filtrates were concentrated under vacuum, dried on open-air for two weeks and kept in a dark place until needed. Aqueous extracts were prepared separately from the fresh powdered sample. A quantity of 50 g of the plant material was mixed with about 300 mL of water and placed in a warm water bath (60°C) for 2 h. After the filtration using normal Whatman filter paper (45 µm), the filtrate was placed in a round-bottomed flask, cooled and coated with dry-ice in acetone. The coated flasks were freeze-dried using the freeze-dryer machine Butchi, LYOVAPOR L-300 for about 24 h. The aqueous dried samples were kept in the dark and at 4°C to prevent re-humidification.

Antimycobacterial activity

Preparation of the broth 7H9 media

Powdered Middlebrook 7H9 broth was dissolved in distilled water and glycerol was added up to 0.2%. The mixture was autoclaved at 121°C for about 15 min and cooled down. A sterile pre-prepared tween 80 (20%) in distilled water was added on the broth media up to 0.25% to prevent clumping and the volume was topped up with the Middlebrook oleic acid-albumin-dextrose (OADC) enrichment up to 10%.

Preparation of plant extracts stock solution

A sufficient volume (10 mL) of plant extract stock solution at 100 mg/mL was prepared. The organic plant extract (100 mg) was first dissolved in 100 µL dimethyl sulfoxide (DMSO) and diluted with 9.9 mL of broth 7H9 media (supplemented with Middlebrook OADC and Tween 80 20%) which brought the DMSO to 1% in the whole solution (Dorin et al., 2001). Aqueous extract stock solutions were prepared with the broth 7H9 media. A volume of 2 to 4 mL of each plant extract was filter-sterilized and stored at 4°C until when needed for experimentation.

Inoculum preparation

A reference strain of *Mycobacterium smegmatis* ATCC607 was used to test the extracts for the antimycobacterial activity. The existing seed stock at the Tuberculosis laboratory, Centre for Respiratory Diseases Research-Kenya Medical Research Institute was diluted in the ratio 1:1000 (v/v) with filter-sterilized broth media in a 750 mL culture bottle and pre-cultured for 16 h at 37°C with no shaking. The pre-grown inoculum was again diluted in the ratio 1:1000 at the time of use (Kigondu, 2015).

Broth microdilution method

The method was carried out according to Collins and Franzblau (1997) with some slight modifications. A two-fold dilution in a 96-well plate was used to determine the minimum inhibitory concentration (MIC99). The broth media 7H9 (50 µL) was added in all the wells, except the first column where the initial concentration of plant extracts was added. Each plant extract was tested in duplicate (100 µL of plant extract solution 100 µg/mL in column 1), with two rows (1st and 8th) serving as negative control and positive control respectively. The two-fold serial dilution was carried out by transferring 50 µL of the content (plant extract + broth media) of the wells of the first column to the next wells until the wells of the 12th column are reached, where 50 µL was aspired off. An aliquot (50 µL) of the pre-grown inoculum (*M. smegmatis* ATCC607) was added in all wells except the row 1 wells serving as the control. The total volume in each well was 100 µL and the initial concentration was diluted to 50 µg/mL by adding the inoculums solution. After sealing the micro plates with parafilm paper, they were placed in a tight box and incubated for 48 h at 37°C.

Freshly prepared resazurin blue dye (20 µL) at 0.01% in distilled water was added and the plates were incubated for another 24 h. The change in color from blue to pink indicated the growth of microorganisms (Inactivity of the plant extract) and the non-color change indicated the inhibition of growth of microorganisms (the activity of the plant extract). Rifampicin was used as the reference standard drug in this study.

Cytotoxicity evaluation of the bioactive plant extracts

The cytotoxicity assessment of the active plant extracts against *M. smegmatis* ATCC607 was carried out according to the MTT-based assay, a method described by Mosmann (1983). Vero cells were grown to confluence. They were trypsinized and the required seeding density of 2.10^5 cells per mL (20,000 cells/ 100 µL) was determined. The cells were seeded (100 µL of the cells suspension) into the 96-well plates and incubated at 37°C for 24 h at 5% CO_2, 80% humidity for the cells to adhere conveniently into the wells. The maintenance media (35 µL) was added in the plates with cells that were incubated the day before, bringing the volume to 135 µL in each well.

An aliquot of 15 µL of the plant extract solution (at 10,000 µg/mL in PBS) was then added in all row H wells. The mixture was homogenized and this brought the total volume per well to 150 µL and the plant extract concentration to 1000 µg/mL. A three-fold serial dilution was performed from row H to row B, with row A serving as a positive control (non-drug-treated cells). The last 50 µL picked from row B was discarded. The micro plates were closed and incubated at 37°C for 48 h at 5% CO_2, 80% humidity. Rifampicin was used as a reference standard drug. For each test sample, two columns were used as a negative control (media and plant extract only) and the tests were carried out in quadruplicates. After 48 h of incubation, 10 µL MTT dye was added to all the wells and the plates incubated for another 4 h (at 37°C at 5% CO_2, 80% humidity) until a purple precipitate was clearly visible in the wells under the light of a microscope. The liquid content of the wells was aspirated off and 100 µL DMSO was added to dissolve the formazan crystals (attached into the wells) produced by viable cells. The absorbance was read using ELISA plate reader at 540 nm with a reference wavelength of 720 nm. The percentage cell viability at different extracts concentration was obtained using this formula:

$$\text{Percentage cell viability} = 100 - \frac{A_t - A_b}{A_c - A_b} * 100$$

Where, At is the absorbance value of the test compound, Ab the absorbance value of the negative control (blank) and Ac the absorbance value of the positive control. The cytotoxic concentration of plant extract which reduces at 50% the Vero cells (CC_{50} value) was estimated using a linear regression equation (Y= aX + b) obtained after plotting the percentage cell viability against drug concentration.

Preliminary phytochemical screening

The phytochemical screening of the active plant extracts was carried out according to Harborne (1984) with minor modifications. Alkaloids were screened using the Dragendorff's reagent, the flavonoids using the alkaline method, phenols by the ferric chloride method, the tannins by the ferric chloride method on a pre-heated sample, the terpenoids by the chloroform-sulphuric acid method and the saponins by the foaming method.

Preparative TLC fractionation

Preparative TLC was carried out on the most active plant extract using a glass plate pre-coated with silica gel 60 PF_{254}, 2 mm of thickness. The sample was loaded continuously from left to right 5 mm above the base of the plate and the best solvent system (Hexane: ethyl acetate in ratio 6: 4) obtained from the TLC experiment was applied. After development, the different bands were scraped off from the plates and dissolved into the most polar solvent of the system, that is, ethyl acetate. The fractions were analyzed further by a Gas Chromatography-Mass Spectrometry (GC- MS) machine.

Gas chromatography-mass spectrometry analysis (GC-MS)

The samples were diluted in ethyl acetate. They were filtered through PTFE 0.45 µm syringe filters and transferred into auto-sampler vials for GC-MS analysis. A Shimadzu QP 2010-SE GC-MS with an auto-sampler was used for the analysis. Ultrapure Helium was used as the carrier gas at a flow rate of 1 mL/ minute. A BPX5 non-polar column, 30 m; 0.25 mm ID; 0.25 µm film thickness, was used for separation. The GC was programmed as follows: 50°C (1 min); 5°C/min to 250°C (4 min). The total run-time was 45

Table 1. Percentage yield of extracts obtained from the selected six plant materials of the 3 plants

Botanical name	Part	% Yield				
		n-hexane	DCM	EtOAc	MeOH	H_2O
C. mildbraedii	Stem bark	1.51	0.18	0.27	7.5	7
	Root bark	2.59	1.65	0.32	3	3
C. edulis	Stem bark	0.21	0.34	0.15	10	24
	Leaves	1.7	1.06	0.42	10	20
C. ellenbeckii	Stem bark	1.69	0.99	0.21	10	10
	Leaves	0.25	0.82	0.57	8.33	18

DCM=Dichloromethane; EtOAc= Ethyl acetate; MeOH= Methanol; H_2O = Water.

min. Only 1 µL of the sample was injected. The injection was done at 200°C in split mode, with a split ratio set to 10:1. The interface temperature was set at 250°C. The electron-ion (EI) source was set at 200°C. Mass analysis was done in full scan mode, 50 – 600 m/z. Detected peaks were matched against the National Institute of Standards and Technology (NIST 2014 MS library) for possible identification.

RESULTS AND DISCUSSION

Extraction percentage yield

The organic solvent and aqueous extractions of the six well-dried plant materials (2 plant parts per plant) led to thirty (30) extracts. Table 1 reports the percentage yield of the extraction with the different solvents. The non-polar and moderate solvents (n-hexane, DCM and Ethyl acetate) extracted more in the root bark (0.32-2.59%) than in the stem bark (0.18-1.51%) for *C. mildbraedii*, while those solvents extracted more in the leaves (0.42-1.7%) than in the stem bark (0.15-0.34%) for *C. edulis*. Hexane and DCM extraction yielded more in the stem bark (1.69% and 0.99%) than in the leaves (0.25% and 0.82%) for the *C. ellenbeckii*. On the contrary, it was not the case for the ethyl acetate extraction.

Extraction using methanol yielded generally comparable quantities of extracts for the different parts of plants under study, except for the *C. mildbraedii* where the yield was higher in the stem bark (7.5%) than in the roots (3%). Moreover, the methanolic extracts represented the major yields compared to others. A comparison between different solvents indicated that methanol is capable of extracting higher quantities and more types of phytochemicals (Truong et al., 2019; Gahlot et al., 2018; Dhawan and Gupta, 2017).

Antimycobacterial activity of *Commiphora* sp. plant materials

The antimycobacterial activities of the 30 plant extracts from the three *Commiphora* species were evaluated using the broth microdilution method. The assay was performed against *M. smegmatis* ATCC607 and eleven (11) out the thirty (30) extracts exhibited antimycobacterial activity (Table 2) with MICs ranging from 0.39 to 50 mg/mL. At least one of the plant parts of the 3 *Commiphora* species under study have showed antimycobacterial activity with *C. mildbraedii* proving to be more active than the other two species i.e. *C. edulis* and *C. ellenbeckii*. The methanolic extracts of *C. mildbraedii* stem bark and root bark, and the aqueous extract of *C. ellenbeckii* showed greater bioactivity with MICs less than 1000 µg/mL (0.39, 0.78, 0.78 mg/mL respectively). The highest MIC (50 mg/mL) was recorded for the DCM extract of *C. edulis* leaves, hence the less active among the tested plant extracts. All the tested plant extracts showed lower activity than the one of rifampicin, the reference drug (MIC= 0.015 mg/mL).

The best bioactivity of the methanolic and ethyl acetate extracts is supported by the qualitative phytochemical screening which proved that those extracts in general, contain phenols, flavonoids, terpenoids, saponins, tannins, and alkaloids (Table 4). Indeed, those bioactive molecules are well known to exhibit antimicrobial activity (Newton et al., 2002; Cushnie and Lamb, 2011, Gupta et al., 2012, Akiyama et al., 2001, Liu and Henkel, 2002; Alves et al., 2013).

Nevertheless, further work for purification of the most active extracts, isolation and testing of the purified compounds are worth to be carried out for a thorough understanding of the active principles behind this antimycobacterial property.

The *Commiphora* genus has been used since time immemorial as an antimicrobial agent (Hanuš et al., 2005). Researchers have assessed several species within this genus for the antimicrobial activity including antibacterial, antifungal and antimycobacterial activity.

The findings obtained from this study can therefore be compared to the findings from previous studies. For instance, *C. edulis* was investigated for the antibacterial

Table 2. Antimycobacterial activity (expressed as MIC_{99}) of the different extracts obtained from the 3 *Commiphora* species under study against *M. smegmatis* ATCC607.

Botanical name	Part	MIC_{99} (mg/mL)				
		n-hexane	DCM	EtOAc	MeOH	H_2O
Commiphora mildbraedii	Stem bark	NA	NA	9.35	0.39	NA
	Root bark	NA	NA	6.25	0.78	NA
C. edulis	Stem bark	NA	1.56	3.125	NA	NA
	Leaves	NA	50	NA	NA	NA
C. ellenbeckii	Stem bark	NA	NA	NA	NA	3.125
	Leaves	NA	NA	12.5	3.125	0.78

NA = Not active at highest tested concentration (50 mg/mL). MIC_{99} for the rifampicin (reference standard drug) = 0.015 mg/mL.

property *Commiphora* species have been evaluated for the antibacterial activity, including *C. myrrha* (Alhussaini et al., 2015), *C. caudata* and *C. berryi* (Latha et al., 2005), *C. molmol* (Abdallah et al., 2009, Kuete et al., 2011, Mohammed and Samy, 2013), *C. gileadensis* (Iluz et al., 2010; Al-Sieni, 2014; Al-Mahbashi et al., 2015), *C. swynertonii* (Mkangara et al., 2014), *C. africana*, *C. shimperi*, *C. grandulosa*, *C. marlothii*, *C. negleta*, *C. pyracanthoides*, *C. tenuipetiola*, *C. veminea* (Paraskeva et al., 2008), *C. guidottii* (Gebrehiwot et al., 2015), *C. pedunculata* (Sallau et al., 2014), *C. kerstingii* (Ibrahim et al., 2016). The antifungal activity was demonstrated in *C. wightii* (Fatopea et al., 2013), *C. kua* (Berzinji et al., 2014), *C. wildii* (Sheehama et al., 2019) and *C. guidottii* (Gebrehiwot et al., 2015).

Four *Commiphora* species so far have been subjected to antimycobacterial evaluation and some of the results obtained are comparable to the ones attained in this study. In a study carried out by De Souza et al. (2017), the chloroform extract of *C. leptophloeos* stem bark was tested against *M. smegmatis* and *M. tuberculosis* and proved to have antimycobacterial activity with MICs of 12.5 and 52 mg/Ml respectively, while the dichloromethane extract of *C. eminii* sap and the sterols isolated from it were tested against *M. madagascariense* and *M. indicus pranii* and showed same activity of MIC at 2.5 and 1.6 mg/mL respectively for the DCM extract and the isolated sterols (Erasto, 2012).

This is comparable to the MIC_{99} (1.56 mg/mL) of the DCM extract of *C. edulis* stem bark obtained in this study. In another study, the *C. mukul* gum was tested against *M. aurum* and showed a MIC of 62.5 µg/ml (Newton et al., 2002), while *C. opobalsamum* essential oils from the fresh aerial parts exhibited good activity (MIC = 15 µg/mL) against *M. intracellulare* (Al- Massarany et al., 2008). The reported results for *C. mukul* and *C. opobalsamum* essential oils are better than the ones obtained from this study. This implies that further studies using essential oils or gum from the *Commiphora* species under investigation here are needed for proper comparison. Nevertheless, the present study demonstrated the antimycobacterial activity of *C. mildbraedii, C. edulis and C. ellenbeckii*, which has not been reported previously.

Cytotoxicity of the active extracts

The MTT-based assay and the Vero cells were used to assess the cytotoxicity of the eleven plant extracts. The CC50 values of the active plant extracts ranged from 339.65 ± 1.38 to 1734.05 ± µg/mL, as presented in Table 3. The active plant extracts tested showed low or no cytotoxicity compared to the reference standard drug rifampicin. The aqueous extract of *C. ellenbeckii* leaves and the ethyl acetate extract of *C. edulis* stem bark presented the lowest cytotoxicity, at CC_{50} of 1509.64 and 1734 µg/mL respectively, compared to rifampicin.

According to Zirihi et al. (2005) and Kigondu et al. (2009), the plant extracts can be considered non-cytotoxic when their CC_{50} values > 20 µg/mL. However, the literature reports that South African *C. edulis* stem bark and leaves chloroform/methanolic extracts exhibited high cytotoxicity (CC_{50} = 194.0 µg/mL and CC_{50} = 99.5 µg/mL respectively) when tested against Graham cells (Paraskeva et al., 2008).

Based on the findings reported here, there is need to carry out further studies to investigate the *in vivo* antimycobacterial activity and toxicity of the *Commiphora* species used in this study.

Preliminary phytochemical screening

Phytochemical screening of various extracts of the plant materials used in this study as presented in Table 4 revealed the presence of alkaloids, phenols, terpenoids, saponins, flavonoids, tannins. The active extracts of *C. mildbraedii* seemed contain similar phytochemicals either in the stem bark or in the root bark with minor variability.

Table 3. Cytotoxicity evaluation (expressed as CC_{50}) of the bioactive plant extracts on Vero cells.

Botanical name	Plant part	Solvent used	Mean CC_{50} (µg/mL)
C. mildbraedii	Stem bark	EtOAc	432.65 ± 9.41
		MeOH	452.80 ± 4.37
	Root bark	EtOAc	339.65 ± 1.38
		MeOH	559.30 ± 35.37
C edulis	Stem bark	DCM	393.54 ± 36.64
		EtOAc	1734.05± 186.04
C. ellenbeckii	Leaves	DCM	506.41 ± 26.08
	Stem bark	Aqueous	448.62 ± 19.00
		EtOAc	420.15 ± 12.59
	Leaves	MeOH	608.53 ± 43.62
		Aqueous	1509.647±67.47
Rifampicin			527.65 ± 48.30

Table 4. Preliminary phytochemical screening of the active plant extracts from the *Commiphora* sp. under studies.

Botanical name	Plant part	Solvent	Parameter					
			Alkaloids	Saponins	Phenols	Tannins	Flavonoids	Terpenoids
C. mildbraedii	Stem bark	EtOAc	+	-	+	-	-	+
		MeOH	-	+	+	-	-	+
	Root bark	EtOAc	+	-	+	+	+	-
		MeOH	-	+	+	+	-	-
C. edulis	Stem bark	DCM	-	-	-	-	+	+
		EtOAc	-	-	-	-	+	-
	Leaves	DCM	-	-	-	-	-	-
C. ellenbeckii	Stem bark	Aqueous	-	+	+	+	-	+
	Leaves	EtOAc	-	-	-	-	+	-
		MeOH	+	+	+	+	+	-
		Aqueous	-	+	+	+	+	+

C. edulis was found to contain a small number of classes of metabolites, at the same time only flavonoids and terpenoids were detected in its active extracts (DCM and ethyl acetate extracts).

The active extracts (aqueous extract of the stem bark and ethyl acetate, methanol and aqueous extracts of the leaves) of *C. ellenbeckii* generally manifested the presence of all the tested phytochemicals. Some or all the phytochemicals detected in the *Commiphora* plants used in this study have been reported present in other species of this genus. These include *C. africana* root bark (Okwute and Ochi, 2017) and stem bark (Nuhu et al., 2016), *C. myrrha* resins (Chandrasekharnath et al., 2013), *C. opobalsamum* aerial parts (Al-Howiriny et al., 2004), *C. caudata* and *C. pubescens*leaves (Deepa et al., 2009), *C. berryi* stem bark (Selvamani et al., 2009), *C. gileadensis* stem bark (Al-Mahbashi et al., 2015), *C. mukul* stem bark and seeds (Singh et al., 2016), *C. kerstingii* leaves (Ibrahim et al., 2016), *C. pedunculata* stem bark (Sallau et al., 2014), *C. guidottii* (Gebrehiwot et al., 2015) and others.

All the detected phytochemicals have been investigated for their antimicrobial properties including antibacterial, antimycobacterial and antifungal activities. Reports from the literature show that alkaloids have antibacterial (Karou et al., 2006) and antimycobacterial (Newton et al., 2002) activities, while flavonoids (Cushnie and Lamb, 2011) and tannins have antibacterial activity (Akiyama et al., 2001; Lerato, 2017). Terpenoids (Gupta et al., 2011), saponins (Liu and Henkel, 2002) and phenols exhibit

antimicrobial activity against a wide range of bacteria and fungi (Alves et al., 2013).

However, it is recommendable to further investigate other phytochemicals that have not been included in this present work. Meanwhile, this is the first time that phytochemical screening of the *C. mildbraedii*, *C. edulis* and *C. ellenbeckii* is being reported to the best of my knowledge.

Preparative thin layer chromatography (p-TLC) and gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract of *C. mildbraedii* stem bark

The most active plant extract (MIC_{99}= 0.39 mg/mL), that is the methanolic extract of the stem bark of *C. mildbraedii,* was further fractionated by p-TLC using hexane-ethyl acetate (6:4) as the solvent system. Seven bands/layers (F1-F7) were obtained and their scraping from the plate led to five reconstituted fractions in ethyl acetate, FF1-FF5 (with F1 and F2 and F6 and F7 pulled together). A plethora of different bioactive compounds were detected in each fraction (Appendix 1; by the GC-MS analysis). In total, 42 different bioactive compounds belonging to various chemical classes were found to be present across the different fractions (Table 5). Two compounds were detected as the most abundant; triterpenoid- lup-20(29)-en-3-one (lupenone) present in FF3 (45.29% peak area) and FF4 (12.6% peak area) and an aromatic compound o- xylene present in FF1 and FF5 at 10% peak area.

The phytoconstituents revealed by GC-MS can be grouped into saturated hydrocarbons-alkanes (12), unsaturated hydrocarbons-alkenes (5), primary fatty alcohols (5), phenols (1), monoterpenoids (2) and triterpenoids (1), indanone (1), aldehydes (2), ethers (2), carboxylic acid (1), carbonate esters (3), fatty acids esters (6) and aromatic hydrocarbon (1). Even though the chemistry of *Commiphora* plants has been thoroughly studied (Hanuš et al., 2005; Shen et al., 2012), the phytoconstituents detected in *C. mildbraedii* stem bark (methanolic extract) are almost all newly reported in this genus, except three compounds including α-terpineol detected in the gum resin of *C. mukul* (Saxena and Sharma, 1998), *C. leptophloeos* essential oils (Da Silva et al., 2015), *C. gileadensis* essential oils (Dudai et al., 2017), *C. wildii* essential oils (Sheehama et al., 2019); tridecene in *C. gileadensis* essential oils (Dudai et al., 2017) and o-xylene in the *C. quadricincta* essential oils (Assad et al., 1997). Researchers have paid more attention and studied the most ethnomedicinally used plant part/product, that is, resinous exudates or gum resin and not the other plant parts i.e. leaves, roots or the entire stem bark (Shen et al., 2012). This is a great contribution so far in the study of *Commiphora* genus chemistry as nobody else has reported on the chemistry of *C. mildbraedii* before, to the best of my knowledge.

Twenty-three compounds out of the 42 have been reported to exhibit antibacterial, antifungal and antimycobacterial activities (Table 5). The antimycobacterial activity of the methanolic extract of *C. mildbraedii* may be due to two of its bioactive compounds which have been reported to possess anti-tuberculosis activity, 1-tetradecene (Kuppuswamy et al., 2013) and 1-heneicosanol (Poongulali and Sundararaman, 2016). Synergism effect of all the antimicrobial phytochemicals may be responsible for the high antimycobacterial activity of the named plant extract under study (Doern, 2014) because the most abundant detected components, that is, lup- 20(29)-en-3-one (Madureira et al., 2003) and o-xylene (Tiwari et al., 2016) are reported to have good antibacterial activity. Thus, the *C. mildbraedii* methanolic extract is also presumably a potential antimicrobial agent against different bacteria and fungi strains based on the reports from the literature on the bioactivities of compounds detected by GC-MS in this study.

Conclusion

The methanolic extract of *C. mildbraedii* stem bark presented the highest antimycobacterial activity and the active plant extracts showed low cytotoxicity. The phytochemical screening revealed that the active extracts of *C. mildbraedii* contain all the tested bioactive phytochemicals except the flavonoids. GC-MS analysis of the p-TLC fractions identified 42 different compounds with 39 compounds being detected for the first time in *Commiphora* genus. Twenty-three (23) of them are reported in the literature to have antimicrobial activities with two particular compounds, 1-tetradecene and 1-heneicosanol being reported to have anti-tuberculosis activity.

The results provided in this study therefore constitute an additional and reliable contribution to the study of the complex and interesting *Commiphora* genus. The findings justify the ethnomedicinal practices of different cultural societies which utilize the *Commiphora* species to treat respiratory diseases including TB.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Table 5. Summary of detected compounds by GC-MS and their reported bioactivities.

S/N	Compounds	Formula	Bioactivity
		Alkanes	
1	2,6,10-trimethyltridecane	$C_{17}H_{36}$	Unknown
2	Decane	$C_{10}H_{24}$	Unknown
3	Cyclooctacosane	$C_{28}H_{56}$	Antibacterial when derivatized (Ali et al., 2011)
4	Dodecane	$C_{12}H_{26}$	Antioxidant and antimicrobial (Nandhini et al., 2015)
5	Heneicosane	$C_{21}H_{44}$	Antioxidant and antimicrobial (Nandhini et al., 2015)
6	Heptadecane	$C_{17}H_{36}$	Antibacterial activity (Uma and Parvathavarthini, 2010)
7	Hexadecane	$C_{16}H_{34}$	Antibacterial activity (Kalpana et al., 2012).
8	Pentadecane	$C_{15}H_{30}$	Cytotoxic activity and antimicrobial (Bruno et al., 2019), antibacterial activity (Uma and Parvathavarthini, 2010)
9	Tetradecane	$C_{14}H_{30}$	Antifungal (Guo et al., 2008) and antibacterial activities (Rahbar et al., 2012)
10	Undecane	$C_{11}H_{24}$	Unknown
11	Nonane, 1-iodo-	$C_9H_{19}I$	Unknown
12	Nonane, 2,2,4,4,6,8,8-heptamethyl-	$C_{16}H_{34}$	Unknown
		Alkenes	
13	1-dodecene	$C_{12}H_{24}$	antibacterial activity (Togashi et al., 2007)
14	1-tetradecene	$C_{13}H_{26}$	Antibacterial, anti-tuberculosis (Kuppuswamy et al., 2013)
15	1-tricosene	$C_{23}H_{46}$	Unknown
16	1-tridecene	$C_{13}H_{26}$	Antibacterial activity (Kumar et al., 2011)
17	Z-5-Nonadecene	$C_{19}H_{38}$	Unknown
		Primary fatty alcohols	
18	1-Heneicosanol	$C_{21}H_{44}O$	Antibacterial and antifungal activities (Arancibia et al., 2016), anti-tuberculosis activity (Poongulali and Sundararaman, 2016)
19	1-heptacosanol	$C_{27}H_{56}O$	Unknown
20	n-nonadecanol	$C_{19}H_{40}O$	Antibacterial activity (Chatterjee et al., 2017)
21	n-tetracosanol	$C_{24}H_{50}O$	Antiproliferative effect (Vergara et al., 2015)
22	n-pentadecanol	$C_{15}H_{32}O$	Antibacterial activity (Chatterjee et al., 2017)
		Phenols	
23	2,4-di-ter-butylphenol	$C_{14}H_{22}O$	Antifungal activity (Sang et al., 2012), antimalarial activity (Kusch et al., 2011)
		Monoterpenoids	
24	Terpin	$C_{10}H_{20}O_2$	Unknown
25	L-α-terpineol	$C_{10}H_{18}O$	Antioxidant, anticancer, anticonvulsant, insecticidal (Khaleel et al., 2018)
		Triterpenoids	
26	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	Anti-HIV properties (Callies et al., 2015), antibacterial activity (Madureira et al., 2003)
		Indanone	
27	1H-Inden-1-one, 2,3-dihydro-	C_9H_8O	Antimicrobial activity (Patil et al., 2017)
		Aldehydes	
28	E-14-Hexadecenal	$C_{16}H_{30}O$	Unknown
29	E-15-Heptadecenal	$C_{17}H_{32}O$	Antibacterial and antioxidant activities (Kumar et al., 2011).

Table 5. Contd.

		Ethers	
30	Ethanol, 2-butoxy-	$C_6H_{14}O_2$	Mild antimicrobial activity ((Bailey and Neihof, 1976)
31	Pentane, 1-ethoxy-	$C_7H_{16}O$	Unknown
		Carboxyilic acids	
32	Pentanoic acid, 3-methyl-4-oxo-	$C_6H_{10}O_3$	Antimicrobial, antioxidant and anticancer activities (Madkour et al., 2017)
		Carbonate esters	
33	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	Unknown
34	Carbonic acid, decyl 2-ethylhexyl ester		Unknown
35	Carbonic acid, 2-ethylhexyl octyl ester	$C_{19}H_{38}O_3$ $C_{17}H_{34}O_3$	Unknown
		Fatty acids esters	
36	Isopropyl myristate	$C_{17}H_{34}0_2$	antioxidant and antimicrobial activity (Chandrasekar et al., 2016, Begum et al., 2016)
37	Isopropyl palmitate	$C_{19}H_{38}O_2$	hepatoprotective and mild anticancer properties (Saxena et al., 2007)
38	Eicosyl trifluoroacetate	$C_{22}H_{41}F_3O_2$	Unknown
39	Heneicosyl heptafluorobutyrate	$C_{25}H_{43}F_7O_2$	Unknown
40	Tetradecyl trifluoroacetate	$C_{16}H_{29}F_3O_2$	Unknown
41	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	Antifungal activity (Ibrahim et al., 2017)
		Aromatic hydrocarbons	
42	O-xylene	$C_6H_4(CH_3)_2$	Antioxidant, antimicrobial and antifungal activities (Tiwari et al., 2016)

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Appendix 1. Compounds detected by GC-MS analysis in different prep-TLC fractions FF1-FF5 of *C. mildbraedii* stem bark MeOH extract.

Prep-TLC fraction	R_f value	GC-MS detected constituents	Retention time	Area (%)
1	0.20-0.33	Pentanoic acid, 3-methyl-4-oxo-	3.05	1.62
		Pentane, 1-ethoxy-	3.24	0.90
		o-Xylene	5.29	9.37
		1-Dodecene	10.07	0.91
		Cyclohexanemethanol,4-hydroxy-α,α,4-trimethyl- or p-menthane-1,8-diol or Terpin	12.33	0.94
		1-Tridecene	13.09	2.02
		Tetradecane	13.18	1.48
		Pentadecane	14.55	0.73
		2,4-Di-tert-butylphenol	14.88	1.37
		Tetradecyl trifluoroacetate	15.77	3.01
		Hexadecane	15.84	1.73
		Heptadecane	17.06	0.92
		n-Nonadecanol-1	18.17	3.29
		Heptadecane	18.22	1.97
		Isopropyl myristate	18.52	1.59
		Nonane, 1-iodo-	19.33	0.92
		n-Nonadecanol-1	20.33	3.17
		Heneicosane	20.37	1.34
		Isopropyl palmitate	20.64	3.91
		1-Tricosene	21.39	2.26
		n-Tetracosanol-1	22.45	3.68
		1-Heptacosanol	25.30	2.46
		Carbonic acid, 2-ethylhexyl octyl ester	27.42	1.46
2	0.47	Pentane, 1-ethoxy-	3.24	1.02
		Ethanol, 2-butoxy-	5.42	1.00
		Undecane	6.86	0.81
		1-Dodecene	10.07	1.01
		Dodecane	10.18	0.65
		1-Tridecene	13.09	1.99
		Tetradecane	13.18	1.45
		2,4-Di-tert-butylphenol	14.88	1.33
		E-15-Heptadecenal	15.77	3.02
		Hexadecane	15.84	1.78
		Heptadecane	17.06	0.66

		Z-5-Nonadecene	18.17	3.16
		Heptadecane	18.22	1.58
		Isopropyl myristate	18.52	1.45
		n-Pentadecanol	19.28	0.57
		Heneicosane	19.33	0.27
		n-Nonadecanol-1	20.33	3.06
		Heneicosane	20.37	1.33
		Isopropyl palmitate	20.64	3.83
		Trifluoroacetoxy hexadecane	21.39	1.38
		n-Tetracosanol-1	22.45	2.64
		1-Heptacosanol	25.30	3.32
		Carbonic acid, 2-ethylhexyl octyl ester	27.41	2.06
3	0.6	Pentane, 1-ethoxy-	3.24	0.52
		Undecane	6.86	0.43
		1-Dodecene	10.07	0.51
		Dodecane	10.18	0.35
		1-Tridecene	13.09	1.15
		Tetradecane	13.18	0.83
		Pentadecane	14.55	0.30
		2,4-Di-tert-butylphenol	14.88	0.80
		E-15-Heptadecenal	15.77	1.73
		Hexadecane	15.84	1.02
		Heptadecane	17.06	0.38
		n-Nonadecanol-1	18.17	1.95
		Heptadecane	18.22	0.96
		Isopropyl myristate	18.53	0.94
		n-Nonadecanol-1	20.33	1.79
		Heneicosane	20.37	0.80
		Isopropyl palmitate	20.64	2.32
		Heneicosyl heptafluorobutyrate	21.39	2.08
		1-Heneicosanol	22.45	1.39
		2,6,10-Trimethyltridecane	22.49	0.33
		Eicosyl trifluoroacetate	25.30	1.39
		Lup-20(29)-en-3-one	30.91	45.29
4	0.73	Pentane, 1-ethoxy-	3.24	0.95
		Undecane	6.86	0.74
		1-Dodecene	10.07	0.87
		Dodecane	10.18	0.73
		L-alpha-Terpineol	10.44	0.73

		1H-Inden-1-one, 2,3-dihydro-	12.01	0.48
		Nonane, 2,2,4,4,6,8,8-heptamethyl-	12.08	0.33
		1-Tetradecene	13.09	1.84
		Tetradecane	13.18	1.26
		Pentadecane	14.55	0.66
		2,4-Di-tert-butylphenol	14.88	1.00
		E-15-Heptadecenal	15.77	2.53
		Hexadecane	15.84	1.45
		Heptadecane	17.06	0.60
		n-Nonadecanol-1	18.17	2.72
		Heptadecane	18.22	1.37
		Isopropyl myristate	18.52	1.28
		n-Pentadecanol	19.27	0.57
		Heptadecane	19.32	0.19
		n-Nonadecanol-1	20.33	2.41
		Heneicosane	20.37	1.16
		Isopropyl palmitate	20.63	3.13
		n-Tetracosanol-1	21.39	1.44
		1-Heneicosanol	22.45	1.21
		Carbonic acid, decyl 2-ethylhexyl ester	27.42	1.65
		Lup-20(29)-en-3-one	30.81	12.60
5	0.8-0.87	Pentane, 1-ethoxy-	3.24	1.10
		o-Xylene	5.29	10.42
		Decane	6.86	0.89
		1-Dodecene	10.07	1.02
		1-Tridecene	13.09	1.67
		Tetradecane	13.18	1.13
		2,4-Di-tert-butylphenol	14.88	1.12
		E-14-Hexadecenal	15.77	2.97
		Hexadecane	15.84	1.82
		n-Nonadecanol-1	18.16	3.00
		Heptadecane	18.22	1.32
		Isopropyl myristate	18.52	1.57
		Carbonic acid, eicosyl vinyl ester	19.32	1.73
		n-Nonadecanol-1	20.33	2.57
		Heneicosane	20.37	1.28
		Isopropyl palmitate	20.63	3.68
		Cyclooctacosane	21.39	4.52
		n-Tetracosanol-1	22.45	3.33