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Anti-Mycobacterial Activity on Middlebrook 7H10 Agar of Selected Congolese Medicinal Plants

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Abstract

In recent years, Tuberculosis (TB) has re-emerged as a serious public health problem worldwide. The disease spreads more easily in overcrowded settings and in conditions of malnutrition and poverty. The emergence of multidrug resistant and lengthy therapy reduces the patient compliance which comprises TB control strategies. In the current study, the leaves of *Terminalia ivorensis, Carapa procera, Fagara macrophylla, Anacardium occidentale, Ficus spp.* and *Drepanoalpha®* were extracted using petroleum ether, ethyl acetate and methanol in order to assess their anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv and *Mycobacterium tuberculosis spp.* on Middlebrook 7H10 agar using a qualitative approach where the activity was determined by the presence or the absence of growth on the plate. The phytochemical screening was used for the identification of the major groups of compounds found in the extracts. The methanolic extract displayed a good activity on both strains than the petroleum ether and ethyl acetate. The presence of alkaloids, flavonoids, tannins, saponins, anthocyanins, quinones known to be of medicinal importance points out a possible source for anti-mycobacterial agents to address the problem of multidrug resistance. The *in vitro* findings of this study provide a partial support for the use of these plants in the management of various infectious diseases as lead to drug discovery and should be reiterated and recommended for a clinical trial using an animal model.

Keywords

Tuberculosis, Middlebrook 7H10 Agar, Anti-mycobacterial Activity, Phytochemical Screening, Medicinal Plants

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1. Introduction

Tuberculosis (TB) remains one of the deadliest communicable diseases with a high morbidity and mortality in human history globally [1, 2]. It is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) and constitutes an important public health concern [3-5]. The global mortality rate stands at two million deaths per year with one third of the world's population infected with TB [6]. In Low-Income countries such the

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Democratic Republic of the Congo (DRC), TB constitutes a challenge where the collapse of health system and the re-emergence of TB along with other infectious diseases and this country is one of the 22 high burden countries [7]. The emergence of drug resistant strains of *M. tuberculosis* is one of the major reasons contributing to the rise of global incidence of tuberculosis since 1980. TB is now a real threat to TB control program in many countries [8-9]. In Africa, there is the largest number of people co-infected with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) and TB. Therefore, HIV is the single most important factor behind Africa's TB re-emergence [10-11]. Since times immemorial, medicinal plants were used for various purposes while in Africa, traditional medicine is of great value and more than 80% of the local African community refer to traditional healers for their primary healthcare needs [12-13]. The medicinal value of these plants lies in some chemical substances such as alkaloids, flavonoids, lignans, fatty acids, polyphenols, quinones, glycosides, carotenoids, steroids, terpenoids, anthraquinones which produce a definite biological action on the human body [14]. In laboratory settings, plant extracts have been shown to have a variety of pharmacological effects like anti-inflammatory, vasodilatory, antimicrobial, anticonvulsant, sedative, antiplasmodial, anti-cancer, antioxidant, anti-sickling, anti-proliferative and antipyretic properties [15]. The main aim of the current research was to assess the *in vitro* anti-mycobacterial property of various extracts (Petroleum ether, Ethyl acetate and Methanol solvents) of *Terminalia ivorensis, Carapa procera, Fagara macrophylla, Anacardium occidentale, Ficus spp*. and Drepanoalpha® using Middlebrook 7H10 agar medium.

2. Material and Methods

2.1. Study Design

The current research used an observational study design in which selected Congolese plants particularly the leaves of *T. ivorensis, C. procera, F. macrophylla, A. occidentale, Ficus spp*. and Drepanoalpha® were used (table 1).

Table 1. Selected medicinal plants used and their local names.

Scientific names	Family	Part used	English names	Local names	References
Terminalia ivorensis	Combretaceae	Leaves	Black afara	-	[46]
Carapa procera Engler	Meliaceae	Leaves	Crabwood	Bula nima, Futi (Kikongo)	[30, 31]
Ficus spp. L.	Moraceae	Leaves	Ficus tree	Arbre qui marche (French)	[44]
Anacardium occidentale L.	Anacardiaceae	Leaves	Cashew	Mbuma ya liboto (Lingala)	[42]
Fagara macrophylla Engler or *Zanthoxylum gilletii* De Wild	Rutaceae	Leaves	Candle wood/ Satin wood	Nkonko, Nkumanga, Nunge nsende (Kikongo)	[47]

Drepanoalpha® is a poly-herbal formula produced through a bio-guided based plant selection by RESUD 'Research for Sustainable Development' of DRC, a scientific NGO consisted of researchers team from University of Kinshasa. This is the result of more than eight years of intensive advanced laboratory research and of different universities which are still carrying out research in the field of sickle cell aneamia [16]. Therefore, crude extracts were prepared and tested *in-vitro* on *M. tuberculosis* H37Rv (slow growth) and *M. tuberculosis spp.* (fast growth) that were obtained from the National Institute for Medical Research (NIMR) in Dar-es-Salaam, Tanzania.

2.2. Plant Collection and Identification

Selected plant species were collected from Gbado-Lité, Nord-Ubangi city in DRC during the dry season between July and August 2014. These plants were authenticated at the department of Biology, Faculty of Sciences, University of Kinshasa and kept in room temperature until use. Thus, the voucher specimens were collected and kept at the Herbarium of the Faculty of Sciences, University of Kinshasa to help for the confirmation of plant identity.

2.3. Extract Preparation

Powders from dried plants were defatted by soaking into petroleum ether (1:10, w/v). The defatted powder was serially extracted by progressively soaking in chloroform, ethyl acetate and methanol in order to increase solvent polarity with occasional shaking. Whatman's n°1 filter paper was used to filter in order to obtain the crude extract. Therefore, the obtained crude solution was concentrated to a minimum volume in a rotary evaporator at 40°C under reduced pressure. The drying, extraction and concentration of extracts were carried out in the Natural Products Laboratory, Department of Chemistry and Industries, Faculty of Sciences, University of Kinshasa, DRC. These extracts were sent to the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture to assess their anti-mycobacterial activity.

2.4. Methods

2.4.1. Middlebrook 7H10 Agar Preparation

The medium was prepared according to the manufacturer's protocol (Becton Dickinson Company (Difco^{TM}), 7 Loveton

Circle, Sparks, Maryland, USA; Lot No 2116212) and was used for the antibiotic susceptibility testing. Then 9.5 g of the powder was suspended in 450 mL of distilled water where five mL of glycerol was added then mixed thoroughly to allow a good mixture. The solution was autoclaved at 121°C during 15 minutes. Having been autoclaved and cooled to 50°C – 55°C, the sterile medium was put in the water bath in order to avoid its solidification. Then, 50 mL of Oleic acid-dextrose catalase (OADC) was added and 20 mL of the liquid medium was pipetted per plate (petri dish).

2.4.2. Antibiotic Susceptibility Test and Anti-mycobacterial Activity of the Extracts Using Proportional Method

The protocol described by Bunalema [8] was used with slight modifications. The two isolates *M. tuberculosis* H37Rv and *M. tuberculosis spp.* were sub-cultured in Lowenstein-Jensen slants for 3 weeks and 4 weeks respectively. The extracts were dissolved in DMSO (to get the desired final concentrations of 20 $\mu g.mL^{-1}$ and 50 $\mu g.mL^{-1}$) and added to the medium (until the tube is half full) before heating at 85°C for 45 minutes in a slanting position in an inspissator (DFT Classic brand) and, it was ready for use after storage at room temperature for 24 h in order to exclude contamination. Tubes containing the medium were inoculated with strains of mycobacteria described above. A stock solution (2 $mg.mL^{-1}$) of selected antibiotics (Isoniazid, Rifampicin and Ethambutol) was prepared respectively. Isoniazid was used as the positive control, while DMSO vehicle was used as the negative control.

The two isolates of *Mycobacteria* were prepared for the antibiotic susceptibility test using the proportional method. Using a 3mm internal diameter wire loop, about 4 mg fresh culture was scraped from LJ medium into in 1 mL of Middlebrook 7H9 broth containing tween 80 in a glass bottle (bijou) with five glass beads and vortexed for about 30 seconds for the homogenization. The suspension was made up to 4 mL by adding 3.5 mL of sterile distilled water and allowed to settle for about 30 min before gently aspirating the upper portion into a fresh bijou bottle to get the suspension. Later, the suspension was further diluted to obtain the turbidity of 0.5 McFarland standard turbidity equivalent to 10^8 cfu using a spectrophotometer. Bacterial suspension was inoculated into extract-free and extract-containing LJ slopes and incubated at 37°C.

Thereafter, 20 mL of the liquid medium was mixed with 20 µL and 50 µL of the extract in a tube then poured in different petri dish. The Petri dish containing antibiotics and extracts were kept in the incubator overnight at 37°C for solidification. After 24 hours, *M. tuberculosis* H37Rv and *M. tuberculosis spp.* were inoculated in both plates along with antibiotics and extracts. These petri dish were then sealed with paraffin and thereafter incubated for eight weeks in the oven, as: + for high growth, ± for moderate growth and – for no growth. The sensitivity of *M. tuberculosis* H37Rv and *M. tuberculosis spp.* to the antibiotics and the extracts were observed by the inhibition appearing in the media. Where there was moderate growth (in terms of number of colonies), the extract was considered to be inhibitory because the growth was less than the negative control. The cultured petri dish were examined visually each week in order to follow up the growth trend. All the experiments were carried out in duplicate due to the slow growth of *Mycobacterium*. The anti-mycobacterial activity test was carried out in the laboratory of Microbiology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, United Republic of Tanzania.

2.4.3. Phytochemical Screening

The phytochemical screening is a chemical screening test that includes a number of qualitative analysis allowing the identification of secondary metabolites present in a certain sample. The detection of these chemical groups is performed through color and precipitation reactions occurring with the addition of specific reagents [17]. The phytochemical screening was carried out according to the standard techniques [18-20].

3. Results and Discussion

3.1. Results

3.1.1. Anti-mycobacterial Activity of Extracts

From the proportional method, the culture plates containing Middlebrook 7H10 agar were examined visually and the majority of sample plates showed no growth in all concentrations of 20 $\mu g.mL^{-1}$ and 50 $\mu g.mL^{-1}$ as well as for the petri dish containing Rifampicin and Isoniazid while there was growth in in the plate containing the ethambutol.

Most of the extracts displayed a good anti-mycobacterial activity against *M. tuberculosis* H37Rv and *M. tuberculosis spp.* The growth was observed at the end of the fourth week at 20 $\mu g.mL^{-1}$ while at 50 $\mu g.mL^{-1}$ the colonies appeared at the end of the fifth week for the negative control. For the antibiotics, both strains were susceptible to rifampicin and isoniazid but showed a resistance to ethambutol though a moderate growth was observed at the beginning of the fourth week. The analysis was qualitative i.e. the activity was based on the absence of colonies in the plate and no quantitative analysis was performed.

The anti-mycobacterial activity of different extracts using Middlebrook 7H10 agar medium is shown in tables 2, 3 and 4 below.

Table 2. Anti-mycobacterial activity of the tested plant extracts with petroleum ether on *M. tuberculosis* H37Rv and *M. tuberculosis* spp. at 20 µg.mL^{-1} and 50 µg.mL^{-1} on Middlebrook 7H10 agar medium.

Plant specimens and antibiotics	Time in weeks															
	1				2				3				4			
	H37Rv		*Mtb spp*		H37Rv		*Mtb spp*		H37Rv		*Mtb spp*		H37Rv		*Mtb spp*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Terminalia ivorensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carapa procera	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Fagara macrophylla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anacardium occidentale	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ficus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drepanoalpha®	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rifampicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoniazid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethambutol	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
(-) control	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	+

Table 2. Continued.

Plant specimens and antibiotics	Time in weeks															
	5				6				7				8			
	H37Rv		*Mtb spp*		H37Rv		*Mtb spp*		H37Rv		*Mtb spp*		H37Rv		*Mtb spp*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Terminalia ivorensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carapa procera	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Fagara macrophylla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anacardium occidentale	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ficus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drepanoalpha®	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rifampicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoniazid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethambutol	+	+	+	-	-	+	+	-	-	+	+	-	+	+	-	-
(-) control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Legend: a: 20 µg.mL^{-1}, b: 50 µg.mL^{-1}, (-): Negative, +: high growth, ±: moderate growth, -: no growth, *Mtb*: *Mycobacterium tuberculosis*

Table 3. Anti-mycobacterial activity of the tested plant extracts with ethyl acetate on *M. tuberculosis* H37Rv and *M. tuberculosis* spp. at 20 µg.mL^{-1} and 50 µg.mL^{-1} on Middlebrook 7H10 agar medium.

Plant specimens and antibiotics	Time in weeks															
	1				2				3				4			
	H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Terminalia ivorensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carapa procera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fagara macrophylla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anacardium occidentale	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ficus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drepano alpha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rifampicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoniazid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethambutol	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
(-) control	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	+

Table 3. Continued.

Plant specimens and antibiotics	Time in weeks															
	5				6				7				8			
	H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Terminalia ivorensis	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Carapa procera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fagara macrophylla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anacardium occidentale	-	-	-	-	+	-	-	-	+	-	-	-	±	-	-	-
Ficus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drepano alpha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rifampicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoniazid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Plant specimens and antibiotics	Time in weeks															
	5				6				7				8			
	H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*		H37Rv		*Mtb spp.*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Ethambutol	+	+	+	-	-	+	+	-	-	+	+	-	+	+	-	-
(-) control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Legend: a: 20 µg.mL^{-1}, b: 50 µg.mL^{-1}, (-): Negative, +: high growth, ±: moderate growth, -: no growth, *Mtb*: *Mycobacterium tuberculosis*

Table 4. Anti-mycobacterial activity of the tested plant extracts with methanol on *M. tuberculosis* H37Rv and *M. tuberculosis* spp. at 20 µgmL^{-1} and 50 µg.mL^{-1} on Middlebrook 7H10 agar medium.

Plant specimens and antibiotics	Time in weeks															
	1				2				3				4			
	H37Rv		*Mtb spp*		H37Rv		*Mtb spp*		H37Rv		*Mtb spp*		H37Rv		*Mtb spp*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Terminalia ivorensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carapa procera	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Fagara macrophylla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anacardium occidentale	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ficus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drepano alpha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rifampicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoniazid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethambutol	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
(-) control	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	+

Table 4. Continued.

Plant specimens and antibiotics	Time in weeks															
	5				6				7				8			
	H37Rv		Mtb spp		H37Rv		Mtb spp		H37Rv		Mtb spp		H37Rv		Mtb spp	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Terminalia ivorensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carapa procera	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Fagara macrophylla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anacardium occidentale	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ficus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drepano alpha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rifampicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoniazid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethambutol	+	+	+	-	-	+	+	-	-	+	+	-	+	+	-	-
(-) control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Legend: a: 20 µg.mL^{-1}, b: 50 µg.mL^{-1}, (-): Negative, +: high growth, ±: moderate growth, -: no growth, *Mtb*: *Mycobacterium tuberculosis*

As shown in (table 2) no growth was recorded on the petri dish cultured with both strains of *Mycobacteria* having the extracts of *T. ivorensis, F. macrophylla, A. occidentale* and *Ficus spp.* as well as for Drépanoalpha®. The growth on the negative control was observed at the beginning of the second week for *M. tuberculosis spp*. At the beginning of the fourth week, a growth was observed on the plate containing *C. procera* cultured with *Mtb* H37Rv at 20 µg.mL^{-1} while a resistance was observed at the third week on the plate containing Ethambutol cultured with *Mtb* H37Rv at 20 µg.mL^{-1} and for *Mycobacterium spp.* at 50 µg.mL^{-1} at the fifth week.

The growth on the negative control was observed at the second week on the plate cultured with *M. tuberculosis spp.* while the plate cultured with H37Rv was observed at the fourth week for both concentrations (table 3). At the fourth week, the growth was observed on the plate containing *T. ivorensis* at 20 µg.mL^{-1} cultured with *Mtb* H37Rv and on *A. occidentale* on *Mtb* H37Rv at 20 µg.mL^{-1} at the sixth week. A moderate growth was recorded on the petri dish containing Ethambutol at the fourth week, and the growth went higher till eight weeks As described in table 4, in plates cultured with *Mtb* H37Rv a growth was recorded only on *C. procera* at 20 µg.mL^{-1} extracted with methanol at the fourth week while a moderate growth was observed with the same extract at 50 µg.mL^{-1}. A resistance was recorded on the plate containing Ethambutol for both strains while the growth on the negative control cultured with *M. tuberculosis* was observed at the second week and for *Mtb* H37Rv the growth was recorded at the fourth week.

3.1.2. Phytochemical Screening

The phytochemical screening was performed using colored and precipitation reactions (table 5).

Table 5. Phytochemical composition of different plants used.

Phytochemical compounds	*T. ivorensis*	*C. procera*	*F. macrophylla*	*A. occidentale*	*Ficus spp.*	Drepanoalpha®
Flavonoids	++	++	++	++	++	++
Anthocyanins	-	++	++	++	++	++
Tannins	++	++	++	++	++	++
Leucoanthocyanins	++	+	++	++	++	++
Bound quinones	++	-	++	++	-	++
Alkaloids	++	++	++	++	++	++
Saponins	+	++	++	++	+	++
Polyphenols	++	++	++	++	++	++

Legend: ++: high concentration, +: low concentration, -: absence of the phytochemical

Flavonoids, tannins, alkaloids and polyphenols were found in high concentration in all extracts while anthocyanins, leucoanthocyanins, bound quinones and saponins were found in high concentration as well but in some extracts. Leucoanthocyanins and saponins were found in moderate concentration in *C. procera* and *Ficus spp.* as well *T. ivorensis* respectively. As well anthocyanins and bound quinones were found in high concentration in some extracts but were found absent in *T. ivorensis*, *C. procera* and *Ficus spp*. respectively.

3.2. Discussion

To assess the the anti-mycobacterial activity of *T. ivorensis, C. procera, F. macrophylla, A. occidentale, Ficus spp.* plant extracts and of *Drepanoalpha*® as the phytomedicine using the qualitative approach using different solvents namely petroleum ether, ethyl acetate and methanol on Middlebrook 7H10 agar medium was the aim of this research. All the extracts assessed have shown a good anti-mycobacterial activity on the medium used where the growth was inhibited 100% during eight weeks. Though, *Mtb* H37Rv grew on the methanolic and petroleum extracts of *C. procera* and the ethyl acetate extract of *T. ivorensis* and *A. occidentale*, their activity can still be considered as effective. Several studies reported the anti-mycobacterial properties of *Terminalia* species but only a few species from this genus have been explored for their anti-mycobacterial constituents. The crude extracts of *T. phanerophlebia* showed good antimicrobial activities against two *Mycobacteria* strains as well as two other bacterial strains responsible for opportunistic infections related to respiratory ailments [21]. *T. ivorensis* is also widely used in traditional medicine in Cote d'Ivoire to treat dermal diseases including local inflammation and can treat voice-loss [22-24]. Although, no study reported the anti-mycobacterial activity of *T. ivorensis;* the findings of the current study can confirm its anti-mycobacterial potential. Nevertheless, it has been confirmed with other species of *Terminalia* genus [25-28].

C. procera showed a good activity on Middlebrook 7H10 agar though *Mtb* H37Rv though a moderate rate was observed. Our previous study reported that this species displayed a good activity on LJ medium than on Middlebrook 7H10 agar [17]. The bark of this plant is used in eye treatments, as genital stimulants, in paralysis, epilepsy, convulsion and spasms as well as for cutaneous and subcutaneous parasitic infections [29]. Meanwhile the leaves are used against malnutrition, stomachache, arthritis, rheumatism, leprosy, pulmonary troubles, venereal diseases [29-31]. Pereira *et al.* [32] reported the anti-malarial of *C. guianensis* [30]. There is limited information regarding the anti-mycobacterial activity of *C. procera* even for other species of the same genus. Henceforth, further studies are necessary for the exploration of this species pharmacological properties.

The anti-mycobacterial activity of the methanolic extract of *A. occidentale* using quantitative approach (BACTEC) was reported by Olugbuyiro *et al.*, [33]. Their report goes along with the current one, though a qualitative approach was used in this research whereby the absence of growth was regarded as effective. Kayoka *et al.* [34] reported the antimycobacterial activity of three species of Anacardiceae against *M. bovis* meanwhile Luo *et al.* [35] also reported the anti-mycobacterial activity of *A. occidentale* against two species of mycobacteria namely *M. smegmatis* ATCC 607 and *M. tuberculosis* H37Rv ATCC 25618. Onasanwo *et al.*, [36] reported the high potent analgesic and anti-inflammatory activities of the leaves of *A. occidentale*. In Brazil and Cuba, cashew leaf and bark tea is used as a douche for vaginal discharge, diarrhea, uterine complaint, dropsy, cholera, stomach disorder, sore throat infections and rheumatism [37-38]. As an edible fruit, it also is used in the treatment of diabetes, weakness, muscular debility, urinary disorders, asthma, eczema psoriasis, cough, intestinal colic, leishmaniasis, venereal diseases as well as impotence and syphilis-related skin disorders and also used as an aphrodisiac [29]. In Bolivia, *A. occidentale* is taken as a brain stimulant to enhance human memory, regarded as a potent diuretic possessing sudorific [38]. *F. macrophylla* is used in the treatment of hypertension, colds and stomachache, fever, malaria, cough, gonorrhea, rheumatism, bilharzia and cancers. Externally, this plant against urogenital complaints including infections and it contributes to the healing of

women womb after childbirth [25, 39]. In addition, branches of this species contain inflammable resins and are used as processional torches by the villagers in West Africa [39].

Ficus spp. and Drepanoalpha® also showed a good anti-mycobacterial activity for all the extracts. Since the very middle age, *Ficus spp.* was used in the treatment ofulcers, splenomegaly, cancer, chronic diarrhea, furuncles, impetigo, dysentery, pains and eczema, rheumatism, lumbago, hemorrhoids, skin diseases, scabies, hiccup, vomiting, leprosy, lower sugar in diabetes, menorrhagia, hemoptysis, gargle for sore throat and used as an aphrodisiac [29, 40]. In the current study, extracts which showed an anti-mycobacterial activity such as *T. ivorensis, C. procera, F. macrophylla, A. occidentale* and *Drepanoalpha*® are less reported in the literature, so further investigations are needed in order to confirm this activity *in vitro* and *in vivo* using an animal model.

Promising leads from plant sources act on newer targets and may play a crucial role in the development of new generation antitubercular drugs [41]. Plants are rich in a wide variety of secondary metabolites providing specific biological properties, and the presence of these chemicals namely alkaloids, flavonoids, tannins, anthocyanins, leucoanthocyanins, quinones, glycosides, carotenoids, steroids, terpenoids, anthraquinones and saponins have been found in most of the plants which have shown the *in vitro* antimicrobial properties [42-43], and can be derived from barks, leaves, flowers, roots, fruits and seeds. The level of each compound can differ from one part to the other of the plant and even among the same species as well to geographical variation [39, 44]. The findings of this study showed the presence of flavonoids, anthocyanins, tannins, leucoanthocyanins, bound quinones, alkaloids, saponins and polyphenols in all the extracts. While anthocyanins and bound quinones were not found in *T. ivorensis*, *C. procera* and *Ficus spp*. respectively. Leucoanthocyanins and saponins were found in moderate concentration in *C. procera* and *T. ivorensis* respectively.

The composition of *C. procera* leaves showed the presence of alkaloids, glycosides, tannins, flavonoids, anthraquinones, alkaloids, saponins triterpenoids, anthocyanins, leucoanthocyanins, quinones and steroids [30-31]. From the leaves of *A. occidentale* the following compounds were identified precisely: alkaloids, oxalates, coumarins, quinones, anthocyanins, triterpenes, tannins, flavonoids and saponins. [45-46]. For *Ficus spp.* as the species is not determined in the present study, a study reported the presence of tannins, flavonoids, saponins, glycosides and alkaloids from the root bark but flavonoids were not found [47-48]. Most of those authors used ethanolic, aqueous and ether extracts and their findings are similar to the current study though the solvents used were not the same. Mpiana, *et al.* [16], reported the presence of phenolic compounds such as anthocyanins, leucoanthocyanins, quinones, tannins, flavonoids terpenoids and alkaloids as well organic acids in Drepanoalpha®. The main observation is that tannins, alkaloids and flavonoids are found in all the parts of the plant namely leaves, stem and root. Ajayi, *et al.* [42], reported that antibiotics or antimicrobial substances like saponins, glycosides, flavonoids, alkaloids and tannins etc. are found to be distributed in all part of the plants. These phytochemicals precisely alkaloids, flavonoids and tannins have been proven to possess anti-mycobacterial activity [8]. Flavonoids were found to inhibit the fatty acids, mycolic acid biosynthesis and *de novo* fatty acid biosynthesis in mycobacteria [41]. The biochemical profiles of a specific plant may be influenced by several factors such as geographical variation, climate, soil, season, and on the other side solvents used, the used part, different methods of extraction have some effects on the composition and diversity of chemicals contained in the medicinal plants [8, 15, 17, 39]. As phytochemicals were identified in different plants used, henceforth the need of identifying and characterizing the molecule(s) that really possess the anti-mycobacterial activity.

4. Conclusion

Most of the selected plants showed a good activity against *M. tuberculosis*, this justifies the reason why these plants are commonly used by herbalists for treatment of various diseases. The findings showed that most of the medicinal plants contain pharmacologically active substances that are anti-mycobacterial. The extracts were found to be generally more active against mycobacteria culture strains on Middlebrook 7H10 agar. The presence of phytochemical compounds points out a possible source for anti-mycobacterial agents to address the problem of MDR-TB and XDR-TB. This study provides a partial support to the use of these plants in the management of various infectious diseases. Further studies are needed in order to assess the *in vivo* activity using animal models and proceed with clinical trials to allow their use in the management of this disease in the community.

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