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Evaluation of antimycobacterial activity of medicinal plants used by Malian traditional medicine practitioners to treat tuberculosis

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ABSTRACT

Global Tuberculosis (TB) control is facing major challenges such as occurrence of multidrug-resistant (MDR) and extensively drug-resistant tuberculosis (XDR). The current TB drugs are getting less effective and associated with side effects limiting their use, especially with MDR and XDR infected patients. In Mali, many medicinal plants are used against various diseases including bacterial infections. The study aimed at studying the antimycobacterial activities of 60 extracts from 22 Malian medicinal. The antibacterial activity against *Mycobacterium tuberculosis* H37Rv was assessed employing micro-broth dilution method. Out of 60 extracts evaluated, eleven from nine different plants were found to be active against H37Rv strain. The minimal inhibitory concentrations (MICs) ranked from 125 µg/mL to 1250 µg/mL. The most active extracts (125 µg/mL) were represented by ethanolic extract of *Saba senegalensis* and *Vitellaria paradoxa* leaves, dichloromethane extract of *Cola cordifolia* leaves, *Strychnos spinosa* and *Ximenia Americana* roots. Ethanolic extract of *Zizyphus mauritiana*, *Guiera senegalensis* and methanolic extract of *Anthocleista djalonensis* also prevented the growth of H37Rv at 250 µg/mL. The results suggest that *Saba senegalensis*, *Vitellaria paradoxa*, *Cola cordifolia*, *Strychnos spinosa* and *Ximenia Americana* could be potential sources of antimycobacterial molecules
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Keywords: Medicinal plants, *Mycobacterium tuberculosis* H37Rv, Mali.

INTRODUCTION

Tuberculosis (TB) is one of the deadliest infectious diseases in the world. There were an estimated 10.4 million new cases of TB, resulting in approximately 1.7 million deaths, and 95% of these deaths occurring in developing countries (WHO,

2016). In Mali the estimated incidence rates of TB were 58 for 100,000 populations with 5809 new cases reported (Togo et al., 2017) The global TB control is facing major challenges, particularly with the phenomenon of TB/HIV coinfection and the increasing rate of multidrug-resistant (MDR) and extensively

drug-resistant (XDR) TB. The current available first-line drugs are hence getting less effective due to this upsurge of drug resistance and the second lines are associated with side effects, which limits their usage (Jnawali and Ryoo, 2013; Floyd et al., 2018). Years ago, the world health organization (WHO) declared that TB is a world urgency and launched several programs to fight the disease, including the search of new remedies and/or new anti-tuberculosis agents (Harries et al., 2018). In spite of the existence of therapeutic regimens, obstacles still remain among others, undesirable effects and toxicities of anti-tuberculosis molecules, tolerance and the increasing resistance of germs to antibiotics thus, leading to the ineffectiveness of these available drugs. Those situations underline the urgency of searching for novel and efficient therapeutic agents.

Most of modern medicines originated from primeval herbal traditions. For their properties of possessing therapeutic values, medicinal plants are employed as medications for human diseases since centuries (Petrovska, 2012; Bashige et al., 2020). Numerous plant products with antibacterial, antiprotozoal and antifungal activities were reported to be useful either for local and/or systemic infections (Arif et al., 2009; Pandey and Kumar, 2013; Donfack et al., 2014). The plant kingdom is an unlimited source of new molecules that can be employed directly as an active ingredient or can serve as a guide molecule for the development of new therapeutic agents. Moreover, plant extracts are considered as potential sources of new anti-tuberculosis drugs (Anochie, 2017; Gupta et al., 2017; Anochie et al., 2018; Ngoufo et al., 2019). The search for new medicines from natural origin with anti-tuberculosis action is therefore an important axis of research.

In Mali, numerous plants are utilized for the treatment of various diseases including bacterial infections by traditional practitioners (Willcox et al., 2012; Sanogo, 2014). The investigation of these traditionally used medicinal plants, is the subject of many works at the department of traditional medicine of the national institute of research and public health (INRSP/DMT) in Mali, in order to improve the

development of traditional medicines in the country.

Therefore, the study aimed at studying the phytochemistry and the inhibitory activity against *Mycobacterium tuberculosis* of 22 local plants used by the traditional healers to “cure” tuberculosis in Mali. We sought here to provide a rational basis for the evaluation of the plant extracts and the determination of their antimycobacterial activities.

MATERIALS AND METHODS

Plant materials

The plant materials consisted of root barks, leaves, gum and stem barks that were collected from 22 different plants in Mali (Table 1). The plants were then authenticated by a botanist at the department of traditional medicine of Mali and an herbarium sheet of each plant was made available at the same department with reference numbers. The collected plants were cleaned with water, air dried under shade and then milled into powder using an electric grinder (Resch grinder type SM 2000 OSI/1400 µm). The powders were weighed with precision analytical balance (type SARTORIUS), and stored in sterile containers with lids at room temperature (± 25 °C) prior usage.

Extraction of plant materials

The extraction of each of the powdered plant materials were performed following the method described previously by Basri and Fan (2005) with few adjustments made. Briefly, maceration by dichloromethane (DCM), 70% ethanol and water (except for the leaves of *D. oliveri* and *S. senegalensis* on which decoction were performed) methods were employed. Powdered Plant material (20 g) was immersed in the corresponding solvents (200 mL) under shaking condition during 24 hours at room temperature. Afterward, the macerates obtained following filtration of the mixture using a compress, were then concentrated in Rotavapor (Büchi R-200). The aqueous and ethanol concentrated extracts obtained from the rotavapor process were frozen and then freeze-

dried, while the concentrates obtained after maceration with DCM were air-dried under hood. All the lyophilized extracts were stored in tightly closed and sterile bottle for testing. The decoction method followed similar procedure; briefly plant powders (20 g) were boiled in distilled water (200 mL) for 15 minutes, filtered after cooling down, then concentrated using the rotavapor. The resulting concentrate was then freeze-dried, and the lyophilized product was stored until further use.

Thin layer chromatography (TLC)

TLC was carried out as described by Bamba et al. (2020) on aluminum plate with support of the silica gel 60 FG₂₅₄ Merck. The Butanol-Acetic Acid-Water (60-15-25) solvent system was used for the aqueous, methanolic and ethanolic extracts; Ether Petroleum-Ethyl Acetate (1-1) was used for DCM extracts and petroleum ether. Plates were observed at 254 and 366 nm UV light. Godin's reagent was used to reveal the components of the extracts.

Mycobacterial strain inoculum preparation

Mycobacterium tuberculosis H37Rv, an American Type Culture Collection strain (ATCC 27294) was used to investigate the antimycobacterial activity of the plant extracts. H37Rv strain was first sub-cultured on solid media (Middlebrook® 7H11 agar) for 14 days, and pure colonies were then inoculated in sterile liquid medium (Middlebrook® 7H9) supplemented with glycerol, Tween 80 and OADC (mixture of Oleic acid, Albumin, Dextrose and Catalase). Inoculum was incubated in a shaking incubator at 37 °C with 5% CO₂ for 4 to 5 days before adjusting the turbidity of the suspension to 0.5 Mac Farland (1.5 × 10⁸ CFU/ml). The adjusted suspension served as inoculum to determine the inhibitory activity of the extracts (Molina-Salinas et al., 2006).

Minimum inhibitory concentration determination by Microplate Alamar Blue Assay

The antimycobacterial activity of the plant extracts was determined using the microplate Alamar Blue Assay (MABA) as previously described by O'Neill et al. (2014) and Molina-Salinas et al. (2006). Briefly, the experimental compounds (plant extracts) were dissolved in 5% dimethyl sulfoxide (DMSO) and then sonicated for 30 minutes to ensure total solubility. Concentrations of 1 mg/mL and 5 mg/mL were used as working solutions. Dilutions of the test extracts and an approved anti-tuberculosis drug (isoniazid, used as standard control drug) were made with varying concentrations. Hundred microliters of each concentration of the plant extracts and the standard drug (isoniazid) were dispensed into the corresponding sterile 96 well microtiter plates with the exception of the wells used for growth control (contain microorganisms and culture media only) and negative control (containing only culture media). *Mycobacterium tuberculosis* H37Rv inoculum was also added to the corresponding 96 wells plates with the exception of the wells used for negative control, final volume in each well were 200 µL.

Plates were covered and sealed with parafilm and incubated at 37 °C for 7 days. At Day 7, 32.5 µL of Alamar blue dye was added to all the 96 wells plates then incubated for 16 to 19 hours at 37 °C in dark. After 16-19 hours, plates were read, and validation of the test was conditioned by oxidation-reduction reaction of Alamar blue dye and bacterial cells indicated by the control wells (growth control, media control, and standard drug control wells). For the tested samples, the blue color is synonymous to lack of bacterial growth and therefore indicating the anti-tuberculosis activity. A turn to pink means bacterial growth. The MIC was defined as the lowest concentration, which prevented a colour change from blue to pink.

Table 1: Malian medicinal plant investigated in this study.

N°	Scientific name	Family	Local name (Bambara)	Plant part used	Reference number
1	<i>Daniella oliveri</i>	FABACEAE	Sanan	Leaves	0190
2	<i>Saba senegalensis</i>	APOCYNACEAE	Zaban	Leaves	0082
3	<i>Securidaca longepedunculata</i>	POLYGALACEAE	Joro	Roots, Leaves	2063
4	<i>Manilkara multinewis</i>	SAPOTACEAE	Koungosumon, koya	Roots	2660
5	<i>Vitellaria paradoxa</i>	SAPOTACEAE	Si	Leaves	2278
6	<i>Entada africana</i>	FABACEAE	Samanéré	Leaves, Stem bark	2368
7	<i>Ostryoderris stuhlmannii</i>	FABACEAE	Muso sana	Leaves	1085
8	<i>Erythrina senegalensis</i>	FABACEAE	N'teblin, n'timini	Roots	2493
9	<i>Guiera senegalensis</i>	COMBRETACEAE	Kunjè, kunyè	Leaves	2345
10	<i>Pteleopsis suberosa</i>	COMBRETACEAE	Tèrèni, ntèlènin	Roots	2335
11	<i>Zizyphus mauritiana</i>	RHAMNACEAE	Ntomono	Roots	2441
12	<i>Ximenia americana</i>	OLACACEAE	Ntonkè	Roots	2389
13	<i>Calotropis procera</i>	APOCYNACEAE	Sukunaji Bara	Roots	0730
14	<i>Moringa oleifera</i>	MORINGACEAE	Bassi yirini	Leaves	1391
15	<i>Strychnos spinosa</i>	LOGANIACEAE	Kule-kule	Root bark	0009
16	<i>Anthocleista djalonensis</i>	LOGANIACEAE	Samâtlo	Stem bark, Leaves	2675
17	<i>Heliotropium indicum</i>	BORAGINACEAE	Nösiku	Leaves	2376
18	<i>Boscia angustifolia</i>	CAPPARACEAE	Berecé	Leaves	2208
19	<i>Opilia celtidifolia</i>	OPILIACEAE	Korôgué	Leaves	2477
20	<i>Crossopteryx febrifuga</i>	RUBIACEAE	Balembo	Stem	2478
21	<i>Cola cordifolia</i>	STERCULIACEAE	N'taba nogo	Leaves, Stem bark	2886
22	<i>Khaya senegalensis</i>	MELIACEAE	Jala	gum	2257

RESULTS

Collection of plants

All the 22 plants included in this study belonged to the phylum *Spermatophytes*, under the branch of *Angiosperms*, class *Dicotyledonous*. They comprise of 13 families (Table 1), where the *Fabaceae* family was the most represented with 4 species followed by the *Apocynaceae*, *Sapotaceae*, *Combretaceae* and *Loganiaceae* families with 2 species respectively. Local names have been assigned to each plant corresponding to the name used to designate the plant by the local population of the place of harvest. Plants are harvested on the basis of their traditional use. The leaves were the most harvested (13 plants) followed by root bark (8 plants), stem bark (4 plants) and gum in one plant. An herbarium of each plant was deposited at the Mali DMT and registered under a given reference number.

Extraction efficiency

Sixty crude extracts were assessed from the 22 plants (9 plants freshly collected, and 33 extracts from 13 other plants were already available at the department of traditional medicine (DMT), Bamako, Mali). Of the 33 extracts provided by DMT, 19 were organic macerations and 14 were aqueous extracts. Out of 19 organic macerations, 10 were ethanolic macerations, 5 were methanolic macerations and 3 were DCM and 1 was petroleum ether macerated. Out of 14 aqueous extracts, there were 11 decoctions, 2 infusions and 1 maceration.

Extraction efficiency had concerned only the 9 plants freshly collected. On each plant part, three different extraction methods were carried out. In general, the best yield was obtained with the ethanolic maceration with *Ximenia americana* (31.85%) which translates the richness of the plant into polar compounds soluble in 70% ethanol and the lowest was obtained with the DCM maceration of *Crossopteryx febrifuga* (0.25%) (Table 2).

Thin layer chromatography (TLC)

TLC allowed characterizing the presence of flavonoids, terpene compounds

and sterols. The flavonoids were characterized in the ethanolic extract of the leaves of *Guiera senegalensis*, *Moringa oleifera*, *Securidaca longepedunculata* and *Daniella oliveri*. The TLC of the low polar extracts showed only the presence of triterpenes. Triterpenes and sterols were characterized in the methanolic extract of leaves of *Moringa oleifera* and *Opilia celtidifolia*, as well as in the ethanolic extract of root bark of *Calotropis procera* and *Strychnos spinosa*. Triterpenes were revealed in all the extracts of *C. cordifolia*, *X. americana*, *V. paradoxa* as well as in the ethanolic and DCM extracts of *S. senegalensis* and *S. spinosa*.

For the fluorescences observed under UV 366 nm not revealed by the Godin. Blue fluorescences are observed with ethanolic extract of stem bark of *C. febrifuga*, root barks of *Z. mauritiana*, and yellow with root barks of *P. suberosa*, *S. spinosa*. Blue and yellow with ethanolic extracts of root bark of *E. senegalensis*, DCM of stem bark of *C. cordifolia*. Red fluorescences were also observed with ethanolic extracts of leaves of *D. oliveri*, *S. senegalensis*, *V. paradoxa*, *S. longepedunculata*, and with methanolic extracts of leaves of *C. cordifolia* and *A. djalonensis*. These yellow, blue, pink fluorescences could be coumarins and the red ones could be anthraquinones.

Antimycobacterial activity of the plant extracts

The finding showed that 19 extracts from 14 plants inhibited the replication of H37Rv strain, where the MICs were ranking from 125 µg/mL to 1250 µg/mL. The most promising MIC values (125 µg/ml) were obtained from the ethanolic extraction of *V. paradoxa* and *S. senegalensis* leaves (Table 4), the dichloromethane extract of *C. cordifolia* leaves, and of *S. spinosa* and *X. Americana* roots (Table 5). Ethanolic extract of *G. senegalensis* leaves, *Z. mauritiana* root and methanolic extract of *A. djalonensis* leaves demonstrated a mycobacterial activity of MIC = 250 µg/mL.

Methanolic and dichloromethane extracts of *Cola cordifolia* stem barks was also be observed to be active at concentration of 312.5 µg/mL, 156.2 µg/mL respectively. Leaves of *O. celtidifolia* Extracted by Dichloromethane was active with a minimal concentration of 625 µg/mL. The highest MIC (1250 µg/mL) was observed with methanolic extract of *C. cordifolia* leaves and with seven aqueous extracts including aqueous infused of *A. djalensis* and *Heliotropium indicum* leaves. In addition, decocted of *O. stuhlmannii*, *S. senegalensis*, *S. longepedunculata* leaves and *Z. mauritiana* root bark (Table 3).

None of the aqueous extract inhibited the growth of *M. tuberculosis* at concentrations below 1000 µg/mL. At the concentration of 1250 µg/mL, 5 extracts performed by

decoction and 2 by infusion inhibited the growth of *M. tuberculosis* H37Rv (Table 3).

The methanolic macerate of the leaves of *A. djalensis* and the stem bark of *C. cordifolia* were active against *M. tuberculosis* as well as the ethanolic macerate of the leaves of *G. senegalensis* and those of the roots of *Z. mauritiana*. The most active were the ethanolic macerate of the leaves of *S. senegalensis* and *V. paradoxa*. Among the polar extracts, the ethanolic extracts were the most active (Table 4).

Among the 12 non polar extracts, 5 extracts by DCM have shown activity against *M. tuberculosis*, the most active being the roots of *Ximenia americana* and *Strychnos spinosa* and the leaves of *Cola cordifolia* (Table 5).

Table 2: Extraction efficiency of plants freshly collected.

Plants (Part used)	Extraction method	Extraction efficiency (%)
<i>Calotropis procera</i> (Root bark)	Aqueous macerate	13.40
	DCM macerate	5.05
	70% ethanolic macerate	7.40
<i>Crossopteryx febrifuga</i> (Stem bark)	Aqueous macerate	12.95
	DCM macerate	0.25
	70% ethanolic macerate	15.1
<i>Daniellia oliveri</i> (Leaves)	Aqueous decoction	19.65
	DCM macerate	4.80
	70% ethanolic macerate	22.60
<i>Khaya senegalensis</i> (Gum)	Aqueous macerate	17.75
	DCM macerate	0.4
	70% ethanolic macerate	1
<i>Pteolopsis suberosa</i> (Root bark)	Aqueous macerate	16.6
	DCM macerate	0.6
	70% ethanolic macerate	19.2
<i>Saba senegalensis</i> (Leaves)	Aqueous decoction	17.55
	DCM macerate	4.5
	70% ethanolic macerate	17.7
<i>Strychnos spinosa</i> (Root bark)	Aqueous macerate	19.95
	DCM macerate	1.55
	70% ethanolic macerate	17.2
<i>Vitellaria paradoxa</i> (Leaves)	Aqueous macerate	10.7
	DCM macerate	6.7
	70% ethanolic macerate	19
<i>Ximenia americana</i> (Root bark)	Aqueous macerate	22
	DCM macerate	1.75
	70% ethanolic macerate	31.85

DCM: dichloromethane.

Table 3: Antimycobacterial activity of aqueous extracts against *M. tuberculosis*.

Scientific Name	Part of plant used	Extraction method	MIC µg/ml
<i>Daniella oliveri</i>	Leaves	Decoction	NA
<i>Saba senegalensis</i>	Leaves	Aqueous decoction	1250
<i>Calotropis procera</i>	Root barks	Maceration	NA
<i>Crossopteryx febrifuga</i>	Root barks	Maceration	NA
<i>Khaya senegalensis</i>	Gums	Maceration	NA
<i>Pteolopsis suberosa</i>	Root barks	Maceration	NA
<i>Strychnos spinosa</i>	Root barks	Maceration	NA
<i>Vitellaria paradoxa</i>	Leaves	Maceration	NA
<i>Ximenia americana</i>	Root barks	Maceration	NA
<i>Securidaca longepedunculata</i>	Root barks	Decoction	NA
<i>Securidaca longepedunculata</i>	Leaves	Aqueous decoction	1250
<i>Manilkara multinewis</i>	Root barks	Decoction	NA
<i>Entada africana</i>	Leaves	Decoction	NA
<i>Entada africana</i>	Stem barks	Decoction	NA
<i>Ostryoderris stuhlmannii</i>	Leaves	Aqueous decoction	1250
<i>Ostryoderris stuhlmannii</i>	Leaves	Maceration	NA
<i>Boscia angustifolia</i>	Leaves	Maceration	NA
<i>Guiera senegalensis</i>	Leaves	Aqueous decoction	1250
<i>Zizyphus mauritiana</i>	Root barks	Aqueous decoction	1250
<i>Moringa oleifera</i>	Leaves	Decoction	NA
<i>Anthocleista djalonenis</i>	Stem barks	Decoction	NA
<i>Anthocleista djalonenis</i>	Leaves	Aqueous infusion	1250
<i>Heliotropium indicum</i>	Leaves	Aqueous infusion	1250

NA: Not active.

Table 4: Antimycobacterial activity of methanolic and ethanolic extracts against *M. tuberculosis*.

Scientific Name	Part of plant used	Extraction Method	MIC (µg/ml)
<i>Calotropis procera</i>	Root barks	Ethanolic macerate	NA
<i>Crossopteryx febrifuga</i>	Root barks	Ethanolic macerate	NA
<i>Daniella oliveri</i>	Leaves	Ethanolic macerate	NA
<i>Khaya senegalensis</i>	Gums	Ethanolic macerate	NA
<i>Pteolopsis suberosa</i>	Root barks	Ethanolic macerate	NA
<i>Saba senegalensis</i>	Leaves	Ethanolic macerate	125
<i>Strychnos spinosa</i>	Root barks	Ethanolic macerate	NA
<i>Vitellaria paradoxa</i>	Leaves	Ethanolic macerate	125
<i>Ximenia americana</i>	Root barks	Ethanolic macerate	NA
<i>Erythrina senegalensis</i>	Root barks	Ethanolic macerate	NA
<i>Securidaca longepedunculata</i>	Root barks	Ethanolic macerate	NA

<i>Securidaca longepedunculata</i>	Leaves	Ethanollic macerate	NA
<i>Zizyphus mauritiana</i>	Root barks	Ethanollic macerate	250
<i>Entada africana</i>	Leaves	Ethanollic macerate	NA
<i>Entada africana</i>	Stem barks	Ethanollic macerate	NA
<i>Ostryoderris stuhlmannii</i>	Leaves	Ethanollic macerate	NA
<i>Guiera senegalensis</i>	Leaves	Ethanollic macerate	250
<i>Moringa oleifera</i>	Leaves	Ethanollic macerate	NA
<i>Opilia celtidifolia</i>	Leaves	Methanollic macerate	NA
<i>Cola cordifolia</i>	Leaves	Methanollic macerate	1250
<i>Cola cordifolia</i>	Stem barks	Methanollic macerate	156.2
<i>Anthocleista djalonenensis</i>	Leaves	Ethanollic macerate	NA
<i>Anthocleista djalonenensis</i>	Leaves	Methanollic macerate	250

NA: Not active.

Table 5: Antimycobacterial activity of dichloromethane (DCM) and ether extracts against *M. tuberculosis*.

Scientific Name	Part of plant used	Extraction Method	MIC ($\mu\text{g/ml}$)
<i>Calotropis procera</i>	Root barks	DCM macerate	NA
<i>Daniella oliveri</i>	Leaves	DCM macerate	NA
<i>Khaya senegalensis</i>	Gums	DCM macerate	NA
<i>Pteleopsis suberosa</i>	Root barks	DCM macerate	NA
<i>Saba senegalensis</i>	Leaves	DCM macerate	NA
<i>Strychnos spinosa</i>	Root barks	DCM macerate	125
<i>Vitellaria paradoxa</i>	Leaves	DCM macerate	NA
<i>Ximenia americana</i>	Root barks	DCM macerate	125
<i>Opilia celtidifolia</i>	Leaves	DCM macerate	625
<i>Cola cordifolia</i>	Leaves	DCM macerate	125
<i>Cola cordifolia</i>	Stem barks	DCM macerate	156.2
<i>Anthocleista djalonenensis</i>	Leaves	Ether macerate	NA
Isoniazide	-	-	0.04

NA: Not active.

DISCUSSION

According to previous studies, polyphenolics, flavonoids, sterols and triterpenoids have some antibacterial properties (Macabeo et al., 2012; Haidara et al., 2020). Plants in this study are sources of these substances and, we examined different crude plants extracts used in Mali by traditional practitioners to treat respiratory infections including tuberculosis.

The antimicrobial activities of crude plant extracts are classified into three different categories based on their MIC values as follow:

significant when the MIC value is less than 100 $\mu\text{g/mL}$, moderate (MIC value comprising in between 100-625 $\mu\text{g/mL}$) and low when the MIC is greater than 625 $\mu\text{g/mL}$ (Simoes et al., 2009; Kuete et al., 2010). In our current study, the extracts were tested against *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294).

In total, 60 extracts from 22 plants were tested, among them 11 extracts from 9 plants inhibited the growth of the *Mycobacterium tuberculosis* strain with MICs ranging from 125 to 625 $\mu\text{g/mL}$. Hence, their inhibitory activity on mycobacterial growth was

considered moderate. Dichloromethane extract of *O. celtidifolia* leaves was the least active with an MIC of 625 µg/mL, which corresponds to the upper limit of the moderately active extracts. Methanolic extract of *Cola cordifolia* stem bark exhibited a MIC of 312.5 µg/mL, as well as that of dichloromethane extract with a MIC of 156.2 µg/ml. Ethanolic extract of *Guiera senegalensis* leaves, *Zizyphus mauritiana* root bark and methanolic extract of *A. djalonensis* leaves inhibited the growth of H37Rv strain with MIC of 250 µg/mL. Study completed by Esimone et al. (2009) showed that the inhibitory activity of methanolic extract of leaves and root of *Anthocleista djalonensis* from against *Mycobacterium smegmatis*, a non-pathogenic strain, was 125 µg/mL.

In our study, the lowest inhibitory concentration (125 µg/mL) was observed with ethanolic extract of *S. senegalensis*, and *V. paradoxa* leaves, the same was observed with dichloromethane extract of *C. cordifolia* leaves and *S. spinosa*, *X. Americana* root barks. Antimycobacterial activity of these extracts could be explained by the presence of polyphenolic substances, flavonoids, sterols or terpene compounds. According to many researchers, these types of compounds may possess antimycobacterial properties. Kuete et al. (2011) showed the inhibitory activity of phenolic compounds (1,7-dihydroxyxanthone, morelloflavone and 7'-O-glucoside of morelloflavone) on *M. tuberculosis*. Besides, Macabeo et al. (2012), showed the inhibitory activity of the chloroformic extract of *Uvaria rufa*, stating that the phytochemical screening showed the presence of terpenoids, sterols and phenolic compounds. The antimycobacterial activity of extracts from these plants had not yet been reported by others.

These preliminary results are encouraging for further deep investigation of active principles on these plants that are active against *M. tuberculosis*.

This also justifies why traditional healers have been using these plants for years. However, it is important to determine the real value of these products in order to utilize them

in regular modern medicine with optimal dose and formulation, which not only provide more options to patients but also prevent drug resistance.

Conclusion

This experimental study carried out at the Mali Department of Traditional Medicine and the HIV/TB research and training center Mycobacteriology laboratory (SEREFO), revealed the chemical compositions as well as the biological activities of plant extracts used by Malian traditional healers for TB treatment. This *in vitro* study showed that Malian plant extracts have antimycobacterial activities, allowing the inventory of plants with potential sources of new antimycobacterial molecules. These results could explain the use of these extracts by traditional healers in the treatment of tuberculosis in Mali. The isolation of flavonoids, sterols and triterpenes could open the door for new research avenues on these active plant extracts.

COMPETING INTERESTS

The authors declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

MB: did the lab work, analyzed the data and commented the results, contributed to the drafting of the manuscript. AMS: contributed to the drafting of the protocol and the manuscript. BD and MS: carried out technical support for bacteriological tests and verified the accuracy of the results. AD and AT: carried out the technical follow-up of the phytochemical tests and provided certain extracts. MM, ENHY, SB and RS: participated in the correction of the manuscript. DD: was the principal investigator. All authors approved the final version of the manuscript

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