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UPLC-MS analyses and evaluation of the antimycobacterial activity of extracts of leaves and stems of *Anogeissus leiocarpus* and *Saba senegalensis* from the north of Ivory Coast

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ABSTRACT

The aim of this work was to identify the main phytocompounds and to assess the antimycobacterial activity of the aqueous and ethanolic extracts of the leaves and stems of *Anogeissus leiocarpus* and *Saba senegalensis* with a view to proving or disproving their use in the treatment of tuberculosis. Evaluation of the antimycobacterial activity showed that the aqueous extract of *A. leiocarpus* leaves exhibited activity against two *Mycobacterium tuberculosis* strains used. This activity could be justified by the presence of polyphenols and alkaloids identified by UPLC-MS analysis. Organ extracts from the two plants identified polyphenols (coumarins, flavonoids, flavonols and quercetin) and alkaloids (hordenine, caffeine and nicotine) with recognised pharmacological properties. Some plants, such as *A. leiocarpus* could be used to treat tuberculosis. A study of the toxicity of the aqueous extract of *A. leiocarpus* leaves will be carried out with a view to producing a drug for treating tuberculosis.

Keywords: *Anogeissus leiocarpus*, *Saba senegalensis*, phytocompounds, *Mycobacterium tuberculosis*, drug.

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INTRODUCTION

Plants have always played an important role in human life for their nutritional and medical needs [1]. *Anogeissus leiocarpus*, a plant species in the Combretaceae family, is a plant used in traditional medicine to treat urinary bilharzia, amoebic dysentery, malaria, trypanosomiasis, ulcers, infantile diarrhoea and haemorrhoids [2-6]. This plant extends geographically from Senegal to Cameroon and Ethiopia [7-8]. *Saba senegalensis* (Apocynaceae), which is a liana of dietary interest, is traditionally used to treat rectal prolapse, otitis, anorexia, dysentery and diuretic wounds [9-11], infectious diseases (lung diseases, boils, diarrhoea), parasites (urinary schistosomiasis), inflammation and headaches [4, 12]. This plant is widespread from Nigeria to Senegal, via Burkina Faso, Ivory Coast, Niger, Guinea and Mali [13]. In view of these medicinal uses, these two plants were selected for chemical and biological investigations with a view to assessing their therapeutic action on tuberculosis. Specifically, the aim was to help establish the chemical composition using Ultra-Performance Liquid Chromatography coupled with a mass spectrometer (UPLC-MS) and to assess the antimycobacterial activity of *Anogeissus leiocarpus* and *Saba senegalensis* organs harvested in the north of Ivory Coast. Tuberculosis is a serious disease caused by *Mycobacterium tuberculosis*, also known as Koch's bacillus [14]. Tuberculosis remains a major public health problem, and is the second leading cause of death from infectious diseases after HIV-AIDS, killing more than two million people every year [15, 16]. Although synthetic anti-tuberculosis antibiotics are available, multi-resistant mycobacteria have been observed, increasing their prevalence [17-18]. In addition, the use of these antibiotics has harmful side effects on the liver [19-20]. Faced with this situation, new solutions need to be found. In this search for solutions, plants with therapeutic uses are increasingly sought after as an alternative for treating infectious diseases [21-22] and for research into bioactive molecules with a view to developing traditional medicines [23-24].

MATERIALS AND METHOD

Material

Plant material

The plant material consists of leaves and stem bark of *Anogeissus leiocarpus* and *Saba senegalensis*. The various organs of the two plants were collected in October 2022 in the town of Korhogo (9° 27‘28’‘North, 5° 37’ 46‘’ West), then authenticated by botanists from the Peleforo Gon Coulibaly University. The various organs were dried for 10 days in a room at room temperature, sheltered from the sun. Finally, the dried organs were crushed in a mortar and sieved to obtain fine powders which were used to prepare the different extracts to be tested.

Laboratory equipment and chemical products

The laboratory equipment consisted of laboratory glassware, an oven, an electronic balance, a refrigerator, MGIT tubes, Nalgene® milipore membranes and an MGIT 960 automated incubator. The chemical equipment consists of ethanol, methanol, acetonitrile and formic acid.

Mycobacterial strains

The two Mycobacterium tuberculosis strains of sensitive quality used came from the Centre for Diagnosis and Research on AIDS and other infectious diseases (CeDReS). They were coded 20EEQS1 and 20EEQS2.

Methods

Extractions

Aqueous extracts

A mass of 7 g of powder from each organ of *Anogeissus leiocarpus* and *Saba senegalensis* was mixed with 70 mL of distilled water. The mixture was boiled at 100°C for 20 min. After filtration, the various decocts obtained were placed in an oven at 50°C for 3 days to obtain the various aqueous extracts. These extracts were used for UPLC-MS analysis and to assess antimycobacterial activity.

Ethanol extracts

A mass of 7 g of each plant powder from the different organs of *Anogeissus leiocarpus* and *Saba senegalensis* was macerated in 70 mL of ethanol for 24 h. After filtration, the different filtrates obtained were placed in an oven at 50°C for 2 days to obtain the different ethanolic extracts. These extracts were also used for UPLC analysis and to assess antimycobacterial activity.

UPLC-MS analysis

Mass spectra were performed using a Waters ACQUITY UPLC-MS instrument consisting of a quadrupole detector, a mass spectrometer equipped with an electrospray ionization interface and a diode array detector. For the tests, 1 mg of each crude ethanolic extract was dissolved in 1 to 1.5 mL of analytical grade methanol. The solution obtained was then filtered and a sufficient quantity of the filtrate was introduced into a small vial designed for the analysis. The analytical solvents used were a binary system of solvents (A; B) with A: H2O/acetonitrile 98/2 + 0.1% formic acid and B: acetonitrile 100% + 0.1% formic acid. The UPLC-MS analysis technique highlights the five majority compounds, which correspond to five peaks on the spectrum of each extract tested. To identify these majority compounds, the crude formulae of several secondary metabolites were filled in according to botanical information. As a result, the appearance of one or more green peaks

confirms the presence of the secondary metabolite recorded in the device, while the appearance of red peaks invalidates its presence.

Antimycobacterial activity

The study of antimycobacterial activity was carried out using the methods described [25-26].

Filtration, sterilization and sterility testing of extracts

100 mg of each extract was suspended in 1 mL of water for injection. The solution obtained was then filtered and sterilized on a Nalgene® milipore membrane (Nalgene® vacuum filtration system filter capacity 500 mL, pore size 0.2 µm). These extracts were then used to perform a sterility test, and those declared sterile were then used to assess anti-bacillary (antimycobacterial) activity.

Strain subculture

Strains coded 20EEQS1 and 20EEQS2 were sub cultured in a liquid culture medium contained in a tube (7 mL MGIT tube) for an average of 5 days' growth. An aliquot of the reference strain stored at -20°C was removed from the library. 500 µl of the isolate, progressively thawed, is inoculated into a medium contained in a tube (7 mL MGIT tube) and previously enriched with OADC in accordance with the manufacturer's instructions from BACTEC DICKINSON. The inoculated tube is incubated in the MGIT 960 automated incubator following the manufacturer's instructions. After automatic detection of in vitro growth, the MPT67 antigen is detected using the immuno-chromatographic test (Bioline MPT67) within 24 hours of detection of in vitro microbial growth.

Measurement of the anti-bacillary activity of sterile extracts

0.8 mL of MGIT 960 growth supplement, 0.1 mL of stock solution of each prepared plant extract and 0.5 mL of test inoculum were added successively to each 7 mL MGIT tube. For each isolate, a growth control tube (Control), containing 0.8 mL of growth supplement (OADC) without antibacterial substance (antibiotic), was made. For the control, 0.1 mL of the test inoculum (reference strain) was first pipetted into 10 mL of sterile physiological water to obtain a 1:100 dilution. Next, 0.5 mL of this test inoculum was added to the control tube. All inoculated tubes were immediately placed in the BACTEC MGIT 960 instrument. The relative growth ratio between the tube containing the plant extracts and the control tube was determined by the BACTEC MGIT 960 software algorithm.

- If the relative growth in the tube containing the extract was equal to or greater than that in the control tube, the isolate was considered resistant to the plant extract;
- If the relative growth is less than that of the control tube, the isolate is considered sensitive to the plant extract.

Statistical analysis

The software algorithm of the BACTEC MGIT 960 system was used to process the antimycobacterial activity results.

RESULTS AND DISCUSSION

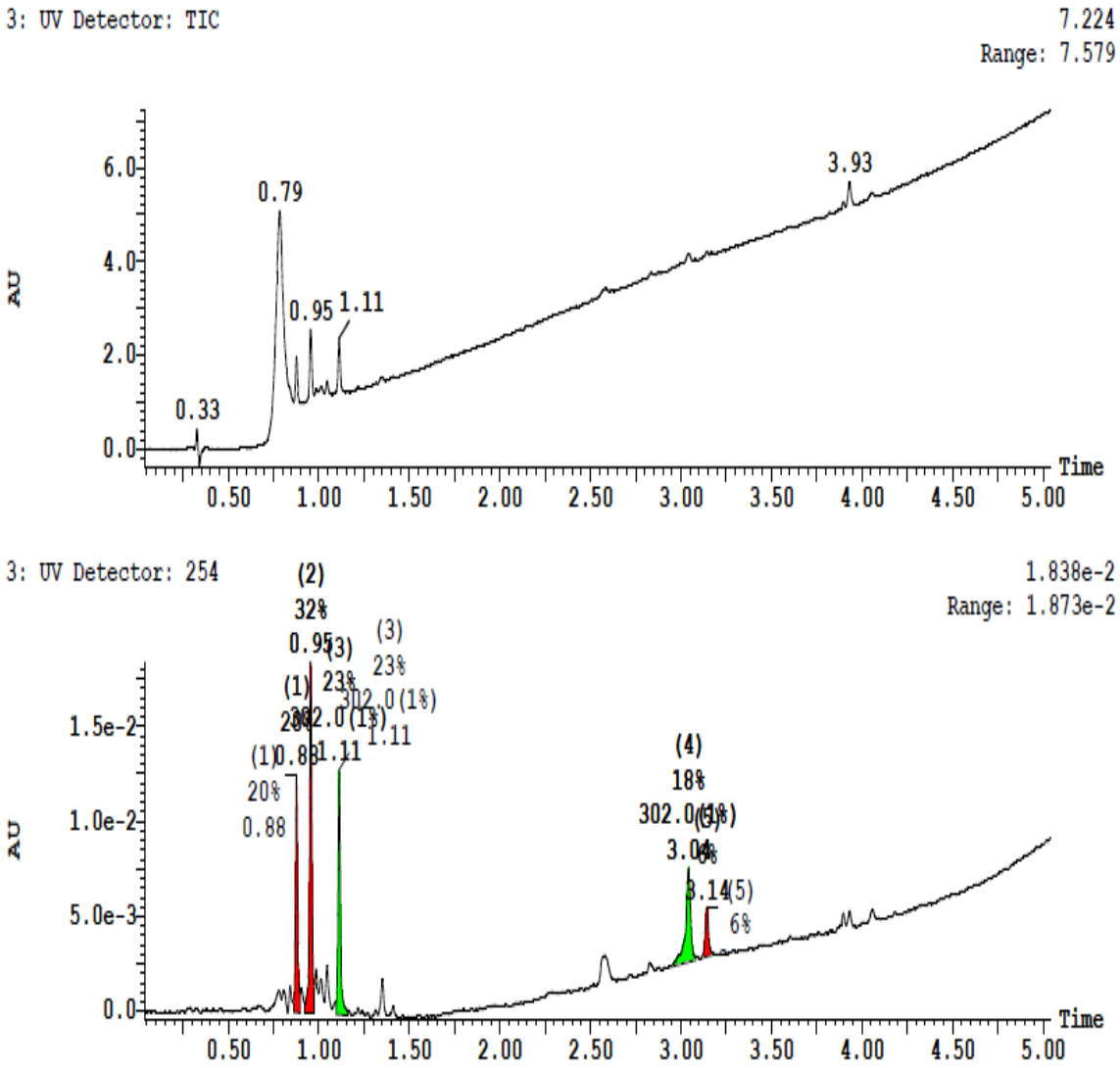

Figure 1: Chromatographic profile of the ethanolic extract of *A. leiocarpus* leaves

UPLC-MS analysis of various aqueous and ethanolic extracts

UPLC-MS analysis of the various extracts of *Anogeissus leiocarpus* and *Saba senegalensis* revealed the five main compounds in each extract (Figure 1). The different chromatographic profiles of the extracts provided information on the retention time (Tr), adsorption wavelength (λ) and percentage presence (P) of each compound. The percentages of presence of the compounds varied from 5.43 to 58.00% and their appearance times varied from 0.69 to 3.93 min. These different characteristics are recorded in Table 1.

Table 1: Characteristics of the five main compounds in the various extracts

		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
ALF1	Tr (mn)	0.79	0.87	0.95	1.11	1.35
	λ (nm)	210.6	213.6	215.6	215.6	220.6
	P (%)	08.45	27.67	29.28	27.58	07.03
ALF2	Tr (mn)	0.88	0.95	1.11	3.04	3.14
	λ (nm)	211.6	210.6	218.6	222.6	222.6
	P (%)	20.42	32.47	22.77	18.05	06.29
ALT1	Tr (mn)	0.78	0.81	0.86	3.04	3.14
	λ (nm)	210.6	211.6	215.6	222.6	222.6
	P (%)	15.93	08.58	44.06	21.12	10.32
ALT2	Tr (mn)	0.81	0.86	0.94	0.95	1.04
	λ (nm)	210.6	215.6	217.6	216.6	214.6
	P (%)	09.91	58.00	13.77	09.16	09.15
SSF1	Tr (mn)	0.69	0.77	2.59	3.05	3.15
	λ (nm)	215.6	210.6	222.6	222.6	222.6
	P (%)	07.08	37.33	12.08	31.87	11.64
SSF2	Tr (mn)	0.77	1.38	1.55	1.77	3.05
	λ (nm)	211.6	217.6	217.6	218.6	222.6
	P (%)	24.14	12.24	09.13	39.64	14.86
SST1	Tr (mn)	2.56	3.03	3.14	3.90	3.93
	λ (nm)	221.6	222.6	221.6	222.6	222.6
	P (%)	19.85	49.48	18.95	05.43	06.28
SST2	Tr (mn)	0.77	2.59	3.02	3.04	3.14
	λ (nm)	210.6	222.6	222.6	222.6	222.6
	P (%)	38.23	12.09	05.43	30.13	14.12

AL : *Anogessus leiocarpus* ; SS : *Saba senegalensis* ; 1 : water ; 2 : ethanol ; Tr retention time of compounds; λ : adsorption wavelength of compounds; P (%) : percentage presence of compounds

Each compound was analysed by comparison with the chromatographic profile of the standard molecules used, and by analysis of the mass spectra and characteristics of each compound. The compounds identified are listed in Table 2 and their structures are shown in Figure 2.

Table 2: Identification of the majority compounds in the various extracts

		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
ALF1	Compound	NI	NI	Flavonol	NI	NI
	Formula			$C_{15}H_{10}O_3$		
	M (g/mol)			238.06		
ALF2	Compound	NI	Hordenine	Quercetin	NI	NI
	Formula		$C_{10}H_{15}ON$	$C_{15}H_{10}O_7$		
	M (g/mol)		165.12	302.04		
ALT1	Compound	Quercetin	Flavonoid		Hordenine	
	Formula	$C_{15}H_{10}O_7$	$C_{15}H_{14}O_5$	NI	$C_{10}H_{15}ON$	NI
	M (g/mol)	302.04	274.08		165.12	
ALT2	Compound	NI	NI	NI	NI	NI
	Formula					
	M (g/mol)					

SSF1	Compound Formula M (g/mol)	NI	NI	NI	NI	NI
SSF2	Compound Formula M (g/mol)	NI	NI	NI	NI	NI
SST1	Compound Formula M (g/mol)	NI	NI	Quercetin $C_{15}H_{10}O_7$ 302.04	NI	NI
SST2	Compound Formula M (g/mol)	Nicotine $C_{10}H_{14}N_2$ 162.12	Caffeine $C_8H_{10}N_4O_2$ 194.08	NI	Flavonol $C_{15}H_{10}O_3$ 238.06	NI

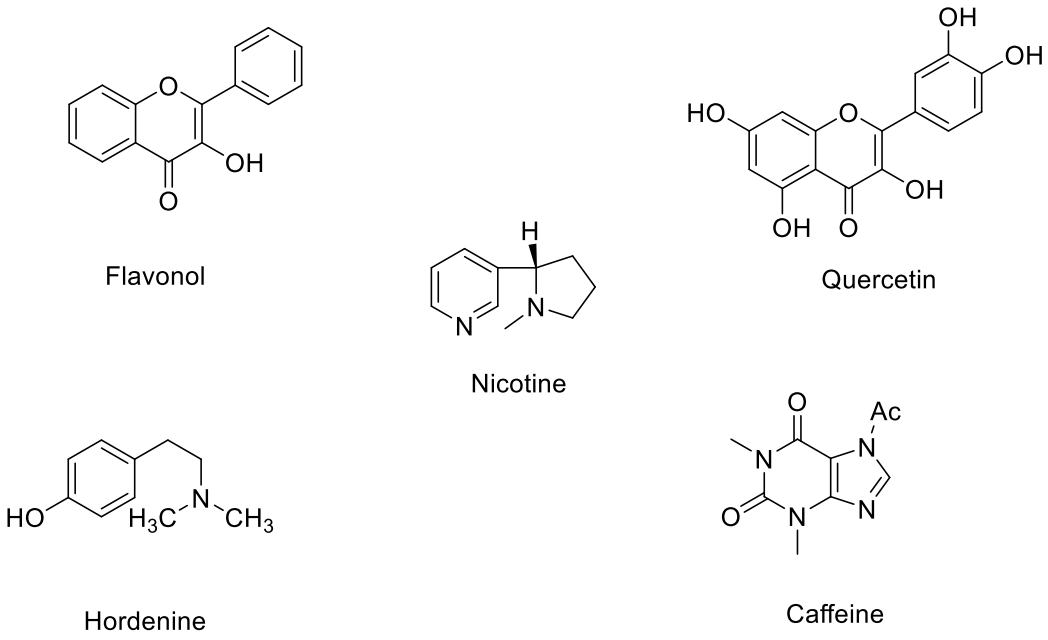

Figure 2: Structures of compounds identified in extracts of *A. leiocarpus* and *S. senegalensis*

Anti-mycobacterial activity of various aqueous and ethanolic extracts

Sterility test for extracts

The results of the extract sterility test are given in Table 3. All the extracts studied were declared sterile except for the ethanolic extracts of *Saba senegalensis*, which contained contaminants.

Table 3: Sterility test results for plant extracts

Extracts	Mass taken	Water for injection	Sterility control on GSC
ALF_1	200 µg	2 mL	Negative
ALF_2	200 µg	2 mL	Negative
ALT_1	200 µg	2 mL	Negative
ALT_2	200 µg	2 mL	Negative
SSF_1	200 µg	2 mL	Negative
SSF_2	200 µg	2 mL	Contaminated
SST_1	200 µg	2 mL	Negative
SST_2	200 µg	2 mL	Contaminated

Assessment of anti-bacillary activity

Only extracts declared sterile were used to assess anti-mycobacterial activity. The anti-tuberculosis effect of the sterile extracts is shown in Figure 3 and the results are reported in Table 4. It was found that only the aqueous extract of *Anogeissus leiocarpus* leaves exerted antimycobacterial activity on the two tuberculosis strains 22EEQS1 and 22EEQS2 used.

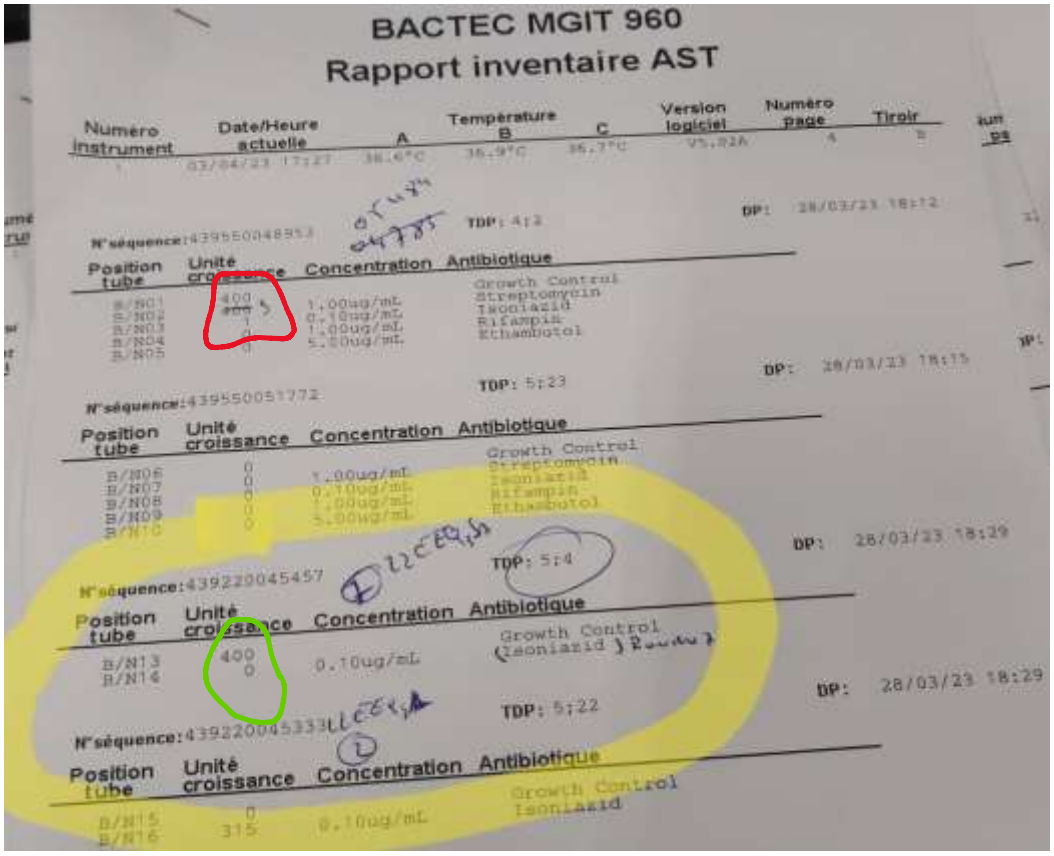

Figure 3: Results determining the inhibitory activity of the aqueous extract of *A. leiocarpus* leaves and the inactivity of *S. senegalensis* on M. tuberculosis after 5.4 days of culture

EPI: water for injection; 22EEQ3S2: reference strain; Reference: Growth Control; Isoniazid: name entered in the MGIT algorithm protocol and for which, read Extract N 3 either SST1

Table 4: Results interpreting the biological activity of extracts on the growth of *M. tuberculosis* 22EEQS1 and 22EEQS2

Extracts (100 mg /mL)	Strain tested 22EEQS1 R/S (UI)	Strain tested 22EEQS2 R/S (UI)	Interpretations
ALF_1	**S (0)**	**S (0)**	**Sensible**
ALF_2	R (400)	R (400)	Resistance
ALT_1	R (400)	R (400)	Resistance
ALT_2	R (400)	R (400)	Resistance
SSF_1	R (400)	R (400)	Resistance
SST_1	R (400)	R (400)	Resistance

R : resistance ; S : Sensitive ; IU : International Growth Unit

DISCUSSION

This work first involved using UPLC-MS analysis to identify the main compounds present in the aqueous and ethanolic extracts of the leaves and stems of *Anogeissus leiocarpus* and *Saba senegalensis*. We then assessed the antimycobacterial activity of these extracts against two tuberculosis strains coded 22EEQS1 and 22EEQS2. UPLC-MS analysis of these extracts was used to record the five main compounds in each extract tested. All of these compounds were present in percentages ranging from 5.43 to 58.00%. UPLC-MS analysis of the extracts from the organs of the two plants detected several phytocompounds. These were alkaloids (hordenine, caffeine and nicotine) and flavonoids (flavonols), including quercetin. Quercetin is the most common flavonoid in the flavonol subclass [27]. The alkaloid family includes hordenine, caffeine and nicotine. UPLC-MS analysis of extracts from the organs of the two plants detected several phytocompounds. These were alkaloids (hordenine, caffeine and nicotine) and flavonoids (flavonols), including quercetin. The presence of these compounds could partly justify the use of these two plants in the traditional treatment of some diseases [2, 3, 4, 5, 6, 9, 10, 11]. Flavonoids have numerous pharmacological properties. They have antiparasitic [27, 28], antibacterial [29], antimicrobial [30], antifungal [31], analgesic [32-33], anti-inflammatory [34], diuretic [30] and antioxidant properties [28]. Quercetin is also one of the best-known flavonoids and is considered to be one of the most potent present in plants [35, 36]. It has been the subject of several scientific reports over the last thirty years. It has multiple beneficial effects on human health, including cardiovascular protection, anti-cancer and anti-ulcer activity, as well as anti-allergic, anti-viral and anti-inflammatory activity [37]. It also has an antibacterial effect [38] and very strong antioxidant properties [36, 39]. Alkaloids are substances of interest because of their pharmacological activities. Although several of them are toxic (strychnine, aconitine), some are used in medicine as analgesics (morphine, codeine), antimalarial agents (quinine, chloroquinine) or anticancer agents (taxol, vinblastine, vincristine) [40-42]. Specifically, caffeine is a compound that acts as a vasoconstrictor and has hypertensive properties [43-44]. Nicotine's secondary effects have a harmful effect on the cardiovascular system. It also has anxiolytic [45] and anti-diuretic properties, and increases blood glucose and fatty acid levels [46]. However, it also plays a role in preventing the development of certain neurodegenerative diseases, such as Alzheimer's and Parkinson's [47]. This was followed by a study of its antimycobacterial activity. This began with a sterility test on the extracts. All the extracts studied were found to be sterile, except for the ethanolic extracts of Saba senegalensis leaves and stems, which were found to be contaminated. This contamination could be due to the equipment used during the extraction and oven concentration of these two extracts. The six other

extracts that were declared sterile were used to assess anti-bacillary activity. At the end of this test, only the aqueous extract of *A. leiocarpus* leaves showed activity after 5.4 days on the two tuberculosis strains 22EEQS1 and 22EEQS2. In fact, the growth inhibitory activity of these strains gave 400 International Units (IU) for the control and zero (0) IU for the strains in the tubes containing the aqueous extract of *A. leiocarpus* leaves. This result shows that plant extracts could be used to treat tuberculosis, especially if they have fewer side-effects than synthetic antibiotics. Indeed, studies have shown that several synthetic antibiotics in general and those used to treat tuberculosis were responsible for liver damage [19]. In addition, Sahli's work on synthetic anti-tuberculosis antibiotics showed that they had harmful effects on the livers of treated subjects. Analysis of these livers revealed a less organized parenchyma and the presence of numerous small fat vesicles in the hepatocytes [20]. The anti-tuberculosis activity of the aqueous extract of *A. leiocarpus* leaves is thought to be due in part to the action of the flavonoids present in this organ. Flavonoids (Flavonol) are generally good antimicrobial agents [28, 30, 48] and in particular formidable antibacterial agents [29, 38].

CONCLUSION

The aim of this work was to identify the main compounds and evaluate the antimycobacterial activity of aqueous and ethanolic extracts of the leaves and stems of *Anogeissus leiocarpus* and *Saba senegalensis*, with a view to proving or disproving their use in the treatment of tuberculosis. UPLC-MS analysis of extracts from the organs of the two plants detected several phytocompounds, including alkaloids (hordenine, caffeine and nicotine) and flavonoids (flavonols), among which quercetin was identified. Evaluation of the antimycobacterial activity showed that the aqueous extract of *A. leiocarpus* leaves showed activity after 5.4 days on the two tuberculosis strains 22EEQS1 and 22EEQS2 used. Some plants such as *A. leiocarpus* could therefore be used to treat tuberculosis, especially if they have fewer side effects than synthetic anti-tuberculosis drugs. A study of the toxicity of the aqueous extract of *A. leiocarpus* leaves will be carried out with a view to producing a drug capable of treating tuberculosis without serious side-effects on human health.

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