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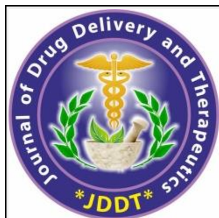

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Open Access Research Article

Phytochemical screening, Antimycobacterial activity of three medicinal Cameroonians plants and Acute toxicity of hydroethanolic extract of *Vitellaria paradoxa*

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex, resposible for health problems in developing countries. In Africa, various medicinal plants are traditionally used to treat TB. The aim of this study is to carry out the phytochemical screening, to evaluate the antimycobacterial activity of the crude extracts of three medicinal plants present in Cameroon (*Zingiber officinale*, *Vitellaria paradoxa* and *Alstonia boonei)* and the acute toxicity of hydroethanolic extract of *Vitellaria paradoxa*. The phytochemical screening was obtained by hydroethanolic extraction and decoction. Inhibitory parameters of antimycobacterial activities were determined using the microplate alamar blue assay against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) and on one *M. tuberculosis* clinical strain. The crude extract with the best antimycobacterial activity was used for the acute toxicity assessment according to the OECD protocol. The results of the phytochemical screening revealed the presence of triterpenes and steroids in all the extracts, whereas phenols were only present in the decoction of *Alstonia boonei*. All extracts tested showed antimycobacterial activities. The hydroethanolic extract of *V. paradoxa* presented the best antimycobacterial activity with MICs of 78.13 and 625 µg/mL and MBCs of 78.13 and 2500 µg/mL respectively on *M. tuberculosis* $H_{37}Rv$ and on *M. tuberculosis* clinical strain. The results of the acute toxicity evaluation of *V. paradoxa* showed a lethal dose 50 greater than 5000 mg/kg compared to control. The antimycobacterial activity of all the plant extracts used in this study justifies the traditional use of these medicinal plants on the treatment of TB.

Keywords: *Zingiber officinale*, *Vitellaria paradoxa*, *Alstonia boonei*, Phytochemical screening, Antimycobacterial activity, Acute toxicity.

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1. INTRODUCTION

Mycobacterium tuberculosis (*M. tuberculosis*), the leading causative agent of tuberculosis (TB) is responsible for the morbidity and mortality of a large population worldwide [1]. It is widespread in poor countries, with more than 80% of cases occurring in Asia and Africa [2]. TB does not exhibit any symptom of disease except when impairment of immunity arises due to malnutrition, diabetes, malignancy and AIDS [3]; however, about 10% of healthy individuals may develop active TB in their life time due to genetic factors. The ability of TB to resist drugs and the influence of HIV epidemy has made the disease remain a devastating global public health problem [4]. In 2016, an estimated number of 10.4 million new TB infections were reported, on which 90% were adults, 65% were male and 10% were people living with HIV [5]. This disease is responsible for approximately two million deaths annually [6]. The alarming rise of multi-drug-resistant (MDR), extensively drug-resistant (XDR) and currently, totally drug resistant (TDR) *M. tuberculosis* strains, which are difficult to control with the currently available essential antitubercular drugs on the market, and

the increased incidence of TB associated with viral infections such as HIV, have recently complicated the chemotherapeutics of TB [7]. Indeed, the side effects of current anti-TB drugs used in long course in combination for the treatment of disease, lead to poor patient compliance and thus promoting the emmergence of resistant strains [8]. As a result, one of the current lines of research is the investigation of new natural compound endowed with antimycobaterial properties and non toxic, which can permit to fight effectively both susceptible and resistant strains of *M. tuberculosis*. Natural products of plant biodiversity have received considerable attention as potential anti-TB agents since they are proven templates for the development of new molecules against TB. Many antitubercular compounds that may prove to be useful leads for TB drug discovery have been derived from medicinal plants [9].

In Africa, many plants are used against various respiratory tract infections, including: *Zingiber officinale* (Zingiberaceae) [10], *Vitellaria paradoxa* (Sapotaceae)[10,11,12] and *Alstonia boonei* (Apocynaceae) [13,14] . Numerous scientific studies have shown that these plants have various biological properties [15, 16, 17], but very few have investigated the antimycobacterial properties of *Zingiber officinale* and *Vitellaria paradoxa* and so far to the best of our knowledge, no study has been carried out on extracts of *Alstonia boonei*.

Ginger (*Z. officinale*) in Cameroon is traditionally used in the villages of the Central Region for the treatment of respiratory diseases [18] and in the Littoral region, for the treatment of infectious disorders, such as pulmonary infections including TB [14]. In Niger, it is used in the treatment of TB and other respiratory diseases [10]. Shea tree (*V. paradoxa*) is traditionally used in Cameroon against intestinal worms in children [19], while in Niger it is used for the treatment of TB and other respiratory diseases [10] and in the states of Bauchi and Ogun in Nigeria for the treatment of TB [11, 12]. As for "devil tree " (*A. boonei*) [20], it is traditionally used for the treatment of endocrino-metabolic, neurological, cardiovascular and infectious disorders such as pulmonary infections and TB [14]. In Kisangani, Congo (Zaire), it is used against throat diseases and TB [13]. However in traditional medicine, the method of preparation commonly used for the use of these plants is the decoction [18]. The purpose of the present study, however, is to carry out the phytochemical screening, determine the antimycobacterial activity and evaluate the acute toxicity of crude extracts obtained by hydroethanolic extraction (30° ethanol) and decoction of *Zingiber officinale*, *Vitellaria paradoxa* and *Alstonia boonei*.

2. MATERIALS AND METHODS

2.1. Chemicals

Rifampicin (Rif) were obtained from Sigma-Aldrich (St. Quentin Fallavier, France). Stock solutions at 1000 µg/mL were filter sterilized and stored at -20°C. Working solutions were prepared at four times the final higher concentration in Middlebrook 7H9 supplemented with 2% glycerol, 10% OADC (oleic acid, albumin, dextrose, and catalase) and 0.05% (vol/vol) Tween 40 (Sigma). Alamar blue dye 0.01% (weight/volume) from Sigma-Aldrich (St. Quentin Fallavier, France) was prepared and sterilized by filtration, and stored at 4°C for up to 1 week. Kit SGM Italia were also obtained from Sigma-Aldrich.

2.2. Collection and extraction of plant materials

Fresh rhizomes of *Z. officinale* were purchased in August 2017 from three Markets (Mvog-beti, Mokolo and Acacia), Yaoundé, Center Region, Cameroon. The stem bark of *V. paradoxa* and *A. boonei* were harvested respectively in September 2017 from Tchollirè village, North Region, Cameroon and in August 2017 from Kala village, Center Region, Cameroon. Botanical identification of the plants was performed at the Cameroon National Herbarium, where the voucher specimens were conserved under the reference numbers 14757/SRF/Cam (*Z. officinale*), 14591/SRF/Cam (*V. paradoxa*) and 1943/SRFK (*A. boonei*).

The fresh rhizomes of *Z. officinale* after being purchased in the different markets were washed with tap water and then cut and dried at room temperature away from light. The barks of *V. paradoxa* and *A. boonei* after harvest were directly cut and dried at room temperature away from light. Once well dried, they were finely crushed manually using mortar and pestle to obtain a powder. The extracts were obtained by two common extraction techniques, maceration and decoction.

According to maceration, The extracts were prepared as earlier described by combination of Prakash *et al.* (2005) [21] and Sourabie *et al.* (2012) [22] with slight modifications. One hundred and fifty grams of each powder were soaked with 750 mL of 30% ethanol in ratio 1:5 (w/v) and mixed intermittently for 48h. This process was repeated three times and subsequently, the suspension was filtered using Whatman No.1 filter paper. The filtrate obtained was concentrated using a rotary evaporator at 79°C, the residue was lyophilized and stored at room temperature.

The extracts obtained by decoction were prepared as earlier described by Sourabie *et al.* (2012) [22] with slight modifications. A total of 1000 mL of boiling distilled water was poured into 150 g of plant powder under constant shaking with magnetic stirrer for 15 min. This operation was repeated twice and at the end of the extraction, it was filtered through nylon mesh followed by Whatman filter paper No.1. The filtrate was then concentrated under reduced pressure on rotary evaporator at 40°C, the residue was lyophilized and stored at room temperature.

2.3 Experimental animals

Albino Wistar rats aged 8 to 12 weeks old, weighing between 125 g to 169 g were obtained from the animal house, of the Department of Animal Biology and Physiology, University of Yaounde I, Cameroon. They were allowed to acclimatize for a period of 2 weeks. They were maintained in clean metabolic cage-sand, placed in a well-ventilated room conditions with a temperature of 26ºC to 28ºC, photoperiods of 12 hours light and 12 hours darkness; humidity of 40% to 60% (Rong *et al*., 2009) [23].

2.4 Phytochemical screening

The various plant extracts were screened for plant metabolites using the lyophilized materials. It was carried out in accordance with standard methods described by Harbone (1998) [24] and Evans (2000) [25]. These metabolites include alcaloid, phenols, polyphenols, tanins, saponins, flavonoids, triterpens, steroids, anthocyanins and anthraquinones.

2.5 *In vitro* antimycobacterial assay

2.5.1 Bacterial strains and growth conditions

The studied microbial species included two strains of mycobacteria, namely, a drug-susceptible strain of *M. tuberculosis H37Rv* (ATCC 27294) and one *M. tuberculosis* clinical strain (MTCS). They were maintained on Lowenstein-Jensen (LJ) slopes and cultured on Middlebrook 7H9 broth supplemented. Cultures were grown aerobically on supplemented broth medium at 37°C.

2.5.2 Microplate alamar blue assay (MABA)

All samples were tested against *M. tuberculosis* using the microplate dilution method [26]. The MIC and MBC of all samples against *M. tuberculosis* strains were tested using the MABA according to Collins and Franzblau, 1997 [27]. Briefly, representative colonies of *M. tuberculosis* from Lowenstein-Jensen (LJ) slope were suspended in 1 mL distilled water and the turbidity was adjusted to match McFarland tube No.1 (10^7 CFU/mL) and further diluted to 1:25 in Middlebrook 7H9 broth supplemented and used as inoculums. One hundred microliters of Middlebrook 7H9 broth supplemented was dispensed in each well of a sterile flat-bottom 96-well plate, and serial twofold dilutions of the crude extracts and each positive control drug were prepared directly in the plate. One hundred microliters of inoculum was added to each well. A growth control and a sterile control were also included for each mycobacterial strain. Sterile water was added to all perimeter wells to avoid evaporation during incubation. The plates were covered, sealed in plastic bags, and incubated at 37°C under a normal atmosphere. After incubation for about 7 days, 20 µL of Alamar blue solution were added to the wells. The plates were re-incubated overnight at the same temperature. A color change from blue to pink indicated bacterial growth. All experiments were carried out in triplicate. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the crude extract or positive control drug that prevented this change in color and the minimum bactericidal concentration (MBC) corresponded to the minimum concentration of extract that did not produce a shift in cultures re-incubated in fresh medium. The classification of the extracts was made by calculating the ratio MBC/MIC as described by Carbonelle *et al.* (1987) [28] and Gatsing *et al.* (2006) [29] for which, an antimicrobial substance is considered bactericidal when the ratio MBC/MIC ≤ 4 and bacteriostatic when the ratio MBC/MIC >4.

2.6 Toxicity test

2.6.1 Acute toxicity study

Acute toxicity study was performed according to the procedures outlined by the Organization for Economic Co-operation and Development guidelines 423 [30]. Twelve Wistar female albino rats were used for this study. The rats were randomly divided into 3 groups of 4 animals each, with the first group as the control. The hydroethanolic extract of *V. paradoxa* was administered to rats in groups 2 and 3 in single oral doses of 2000 mg/kg and 5000 mg/kg body weight respectively, by intra gastric gavage using oral cannula. The control group (Group 1) received an equal volume of distilled water. Observations of toxic symptoms were made and recorded within the first hour, four hours and subsequently for 24 hours after administration of the extract. Behavioral parameters and mortality were also monitored closely for 14 days. Lethal dose in 50 % of the total population (LD50) was interpolated using OECD method [30].

2.6.2 Body weight

The rats in all the groups were weighed using a sensitive balance, before starting the administration of extract, every two days during the observation period and once on the day of sacrifice. Doses of the extract administered were adjusted accordingly.

2.6.3 Relative organ weight

On day 14 of the observation period, all the animals were euthanised by exsanguination under chloroform anesthesia. The rats were later sacrificed through lumbar dislocation. Different organs namely the kidneys, liver, spleen, lungs and heart were carefully dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows:

$$\text{Relative organ Weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body Weight of rat sacrifice day (g)}} X100$$

2.6.4 Biochemical analyses

Blood samples were collected from the animals through cardiac puncture, into clean and dry tubes. The blood collected was allowed to clot and then centrifuged at 5000 rpm for 10 minutes. The serum was separated and kept frozen at -4ºC until required for analysis (Aniagu *et al*., 2005) [23]. The separated clear sera collected were used for the biochemical assays of AST, ALT and Creatinin using SGM Italia reagent kit.

2.7 Statistical analysis

The experimental results were expressed as the Mean ± S.E.M. Statistical significant difference in parameters amongst groups was determined by One way ANOVA followed by Tukey's multiple range test using GraphPad version 7.0. $P<0.05$ was considered to be significant.

3 RESULTS AND DISCUSSION

The extraction yields and the physical properties of the various extracts obtained by hydroethanol maceration and decoction are recorded in Table I. These yields ranging from 5.97 to 13.63% respectively for *V. paradoxa* bark and *Z. officinale* rhizomes for maceration. Meanwhile, in decoction they ranging from 2.36 to 27.77% respectively for *A.boonei* bark and *Z. officinale* rhizomes. For two (02) of the three (03) samples, the extraction yield obtained by decoction is greater than that obtained by hydroethanol maceration for a similar sample. These results corroborate those of Mahmoudi *et al.* (2013) [31] and Lehout and Laib (2015) [32], who made the same observation in their work. However, for *A.boonei* bark, the extraction yield by hydroethanol maceration was higher than that of the decoction. This reduction in the extraction yield of *A. boonei* by decoction would probably be due to the denaturation of certain thermosensitive compounds by heat, as presented by the work of Kémajou *et al.* (2012) [33], who evaluated the influence of drying temperature on the active ingredients (total alkaloids) of *A. boonei*; where they found that the total alkaloids content of the bark extract gradually decreases as the drying temperature increases. In addition, rhizomes of *Z. officinale* had the best extraction yield, both in hydroethanol maceration and decoction. This observation could be explained by the fact that rhizomes of *Z. officinale* contain more active compounds. It is in the same light that the work of Mariangela *et al*. (2015) [34], demonstrated that in addition to the pulp of rhizomes of *Z. officinale*, the skin of this latter also contains active compounds and that it contains more non-polar compounds and even twice as many polyphenolic compounds as the pulp. In fact, according to Bruneton (1999) [35] the extraction yields of the plants are different depending on the species of the plant, the organ and the extraction solvent. The determination of the extraction yield makes it possible to assess the total extract of each species and organ of the plant thus making it possible to estimate the quantity of the part of the plant to be harvested if necessary to allow the rational use of these plant species [36] .

Table I: Extraction yield and physical properties of the extracts

Extraction types	Extraction solvents	Extract types	Extraction yield (%)	Physical properties of extracts obtained	
				Color	State
Extraction by Maceration	Hydroethanol (70/30, v/v)	M Z. o	13,63	Yellow	Liquid
		M V. p	5,97	Brick red	Liquid
		M A. b	10,97	Pale yellow	Liquid
Extraction by Decoction	Distilled water	D Z. o	27,77	Yellow	Viscous
		D V. p	10,36	Brick red	Liquid
		D A. b	2,36	Yellow	Liquid

Legend: M = Macerat; D = Decoction; Z. o = *Zingiber officinale* ; V. p = *Vitellaria paradoxa* ; A. b = *Alstonia boonei*

The results of the phytochemical screening of the extracts are shown in Table II. From this phytochemical test, it is seen that all the extracts contain triterpenes and steroids, and that among these extracts, only the decoction of *A. boonei* contains phenols. This could be explained by the fact that during extraction, the heat produced during the decoction would have caused the breaking of certain bonds, thus causing the formation of phenols. This is in line with results obtained by Ross *et al.* (2011) [37] who reported that under the effect of high temperature, there is partial degradation of lignin, leading to the release of phenolic acid derivatives. Amongst other things, saponins would probably be sensitive to heat because their presence is considerably reduced, or even absent in decoction compared to maceration in all samples. Alkaloids, saponins, flavonoids and steroids present in *Z. officinale* extract have also been reported in the work of Kobo *et al.* (2014) [38]. Amongst others, Amari (2016) [39] in his work highlighted the presence of flavonoids, triterpenes and the absence of anthraquinones in *Z. officinale* extract as presented by the results obtained. In addition, Ogudo *et al.* (2014) [40] also reported in their work the presence of alkaloids, flavonoids and the absence of phenols as observed in the present work, but the absence of saponins and anthraquinones in their extract does not agree with the results here obtained. On the other hand, the absence of tannins in the extract presented here runs counter to the results obtained by all of them. However, their study did not indicate the presence of polyphenols and anthocyanins which were rather present in this study. This test permitted us to reveal the presence alkaloids, saponins, flavonoids, triterpenes and steroids in *A. boonei* extract. These results concords with those of Voukeng *et al.* (2016) [41] and Opoku and Akoto. (2015) [42], who used the bark and roots of *A. boonei* respectively. Nevertheless, the presence of anthraquinones reported by these works are contrary to those of Opoku and Akoto. (2015) [42]. Owolabi *et al.* (2014) [43] also reported the presence of alkaloids, saponins, flavonoids, anthraquinones and the absence of tannins in *A. boonei* leaves just as those obtained with the bark in this study. None of the previous studies tested for presence of phenols and anthocyanins which were however absent and present respectively in this study. The presence of alkaloids, tannins, saponins and the absence of phenols evidenced by this phytochemical test in *V. paradoxa* bark extract coroborates with the results obtained by El-Mahmood *et al.* (2008) [44], while the anthraquinones present in this extract were absent. These latter did not search for polyphenols, flavonoids, triterpenes, steroids and anthocyanins, which were all present in this study. In addition, Ndukwe *et al.* (2007) [45] noted the absence of flavonoids in their extracts, which is contrary to that of this work and the presence of alkaloids, saponins and steroids, which is similar to the results obtained in this study. However, Fodouop *et al.* (2017) [46] showed the presence of alkaloids, polyphenols, tannins and flavonoids in the leave extract of *V. paradoxa* similar to that in this study. On the other hand, the presence of phenols and the absence of saponins, triterpenes, steroids, anthocyanins and anthraquinones reported in their results do not agree with those presented here. The difference in chemical composition of the extracts presented by the results of this work and others can be explained by the nature of the solvent, the part of the plant used, the technique of extraction and the nature of the soil on which the plant has grown, as reported by several authors such as Bruneton (1999) [47] and Nyegue (2006) [48], who have demonstrated that the chemical composition of plant Essential oils can vary depending on the climate and the nature of the soil. In addition, Hudaib *et al.* (2002) [49] also indicated that theperiod of collection, method of conservation, genetic factors and vegetative cycle of the plant can also influence the variability of the chemical composition.

Table II: Phytochemical Screening

Families of secondary metabolites	Crudes extracts					
	Hydroehanolic Maceration			Decoction		
	MZ. o	MV. p	MA. b	DZ. o	DV. p	DA. b
Alkaloids	++	+	+++	+/-	-	+
Phenols	-	-	-	-	-	++
Polyphenols	+	+	+	++	+	+/-
Tanins	-	++	-	-	++	+/-
Saponins	++	+	+	-	-	-
Flavonoids	+/-	++	+	-	++	+/-
Triterpenes	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Anthocyanins	+	++	++	+	++	+
Anthraquinones	-	+	+	+/-	+	+

Legend : - : absent; +/- : presence as a trace; + : weak presence ; ++ : average presence ; +++ : high presence

The antimycobacterial tests on *M. tuberculosis* $H_{37}Rv$ and MTCS made it possible to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) and the MBC/MIC ratio on these latter and grouped respectively in Tables III and IV. On *M. tuberculosis* $H_{37}Rv$, the best MIC of 78.13 µg/ mL also representing the best MBC was obtained with the bark of *V. paradoxa* both with maceration and decoction. The classification of these extracts shows that, only the extract of *A. boonei* obtained by hydroethanolic maceration is bacteriostatic. However, the MIC of 1250 µg/mL obtained with *Z. officinale* extract on *M. tuberculosis* $H_{37}Rv$ is eight times lower than that reported by Nguta *et al.* (2016) [50] on the same strain that was 10,000 µg/mL. Furthermore, Ballo (2013) [19] in his work with *V. paradoxa* leaves obtained via a 70° ethanol maceration of this latter, a MIC of 125 µg/mL on $H_{37}Rv$. The present study with the bark of this latter by maceration with ethanol 30° revealed a MIC of 78.13 µg/mL on the same strain. On the other hand, on the MTCS, the best MIC (625 µg/mL) and MBC (2500 µg/mL) was obtained with the hydroethanolic extract of *V. paradoxa*. All these extracts were bactericidal on the MTCS, except the decoction of *Z. officinale* which could not be classified considering that its MBC could not be determined (it did not belong to the range of the concentrations). The significant difference between the results obtained in this study and those of the other studies could be explained on the one hand by the difference in the chemical composition of plant organs, which is a function of different factors such as climate, soil type [47,48], the period of collection, the method of conservation, genetic factors and the vegetative cycle of the plant [49] and on the other hand the nature of the solvent used Paiva *et al.* (2010) [51] showed that, depending on the polarity of the extraction solvents, the metabolites present in the extracts differ in their polarity and structure and hence, the antimicrobial activities of the compounds extracted from plants changes with a change in structure. It is in this sense that Cowan (1999) [52] alluded to the example between catechol and pyrogallol, both of which are hydroxylated phenols that have been shown to be toxic to microorganisms. Catechol has two hydroxyl groups and pyrogallol has three hydroxyl groups. The site (s) and number of groups on the aromatic ring are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation leads to increased toxicity. In addition, some authors have found that more strongly oxidized phenols are more inhibitory[52]. It also appears from the results of this work that, in general, the hydroethanolic extracts were more active than those obtained by decoction on the two strains. Nevertheless, despite the fact that extracts obtained by decoction generally contain ~~with~~ more chemical compounds due to their high yield, they are still less active than hydroethanolic extracts. This could be explained by the difference in concentration of active ingredients in plant extracts [53]. Indeed, decoction despite their high yield contain a low concentration of active ingredients with antimycobacterial properties, compared to extracts obtained by hydroethanolic maceration, which for their low yield contain a large number of compounds with antimycobacterial properties. This low decoction activity can also be explained by the fact that the antimycobacterial activity of the compounds in these extracts would be masked by other compounds present in the extract [54]. According to Kuete (2010) [55] the activity of plant extracts can be classified on the basis of MICs obtained in antimicrobial tests. Thus, it can be: significant (MIC <100 µg/mL), moderate (100 <MIC <625 µg/mL) or low (MIC> 625 µg/mL). In this rank of idea, among the hydroethanolic extracts, the activity was significant for the bark of *V. paradoxa* (MIC = 78.13 µg/mL), moderate for the bark of *A. boonei* (MIC = 156 µg/mL) and low for rhizomes of *Z. officinale* (MIC = 1250 µg/mL) on *M. tuberculosis* $H_{37}Rv$. In addition, with decocts on *M. tuberculosis* $H_{37}Rv$, the activity was significant for the bark of *V. paradoxa* (MIC = 78.13 µg/mL), low for the bark of *A. boonei* and rhizomes of *Z. officinale* (respectively at MICs of 2500 and 5000 µg/mL). On the other hand, on the clinical strain of *M. tuberculosis*, the activity of hydroethanolic extracts was moderate for bark of *V. paradoxa* (MIC = 625 µg/mL) and low for all other extracts (with MICs of 2500 and 5000 µg/mL respectively for *A. boonei* and *Z. officinale* in hydroethanolic maceration while in decoction, MICs were 1250, 5000 and 10,000 µg/mL respectively for *V. paradoxa*, *A. boonei* and *Z. officinale*). Seen these results, this clinical isolate would probably be resistant. Indeed, by observing MIC obtained on this isolate with the reference anti-TB drug (Rifampicin), which was 3.91 µg/mL this result goes in the same line as that of Gordien *et al.* (2009) [56] who in their study obtained a MIC greater to 3.29 µg/mL on a Rifampicin-resistant isolate. This seems to confirm the resistant character of this clinical isolate and in particular resistant to Rifampicin. Amongst others , all decocts had a weak activity on the clinical strain. The antimycobacterial activities of these extracts could be explained by the presence of alkaloids, phenols, polyphenols, flavonoids, quinones, terpenoids and steroids (Brent, 2003) [57]. However, Kuete *et al.* (2010) [55] showed the inhibitory activity of flavonoids of *Dorstenia barteri* on *M. tuberculosis*, while Gordien *et al.* (2009) [56] revealed the antimycobacterial activities of terpenoids *Juniperus communis* L on *M. tuberculosis*. In addition, Kuete *et al.* (2009) [58] presented the inhibitory activity of naphthoquinones from two species of *Diospyros*, *Diospyros canaliculata* and *Diospyros crassiflora* on *M. tuberculosis*. As a result, anthraquinones belonging to the same family as naphthoquinones and having the same functional group would probably also have a role in the antimycobacterial potential of the extracts where they are present. The antimycobacterial activity exerted by rhizome extracts of *Z. officinale* is probably due to the gingerol contained in these rhizomes [59]. Amongst others, Moustafa *et al.* (2016) [17] reported the presence of three phenolic compounds quercetin, catechin and epicatechin in the bark of *V. paradoxa* while the work of Raju *et al.* (2015) [60] showed the inhibitory activity of quercetin and epicatechin on *M. tuberculosis*. This could explain the antimycobacterial activity of *V. paradoxa* bark extracts in this study. So far, to the best of our knowledge, no scientific study has evaluated the antimycobacterial properties of *A. boonei*, however Adotey *et al.* (2012) [61] recalled in their works that bark of *A. boonei* were isolated from alkaloids, the major ones being echitamine and echitamidine and triterpenoids such as lupeol, ursolic acid and β-amyrin. These metabolites are thus likely to be responsible for the antimycobacterial activity of this latter.

Table III: Activity levels (MIC and MBC), MBC/MIC ratio and extracts classification against *M. tuberculosis* $H_{37}Rv$

Extracts		Activity levels against *M. tuberculosis* $H_{37}Rv$ (µg/mL)		Ratio MBC/MIC	Classification of extracts
		CMI	**CMB**		
Hydroéthanolic Maceration	$M_{Z.o}$	1250	5000	4	Bactericidal
	$M_{V.p}$	78.13	78.13	1	Bactericidal
	$M_{A.b}$	156.25	2500	16	Bacteriostatic
Decoction	$D_{Z.o}$	5000	5000	1	Bactericidal
	$D_{V.p}$	78.13	78.13	1	Bactericidal
	$D_{A.b}$	2500	5000	2	Bactericidal
Rifampicin		0.49	0.49	1	Bactericidal

Table IV: Activity levels (MIC and MBC), MBC/MIC ratio and extracts classification against the MTCS

Extracts		Activity levels against **MTCS (µg/mL)**		Ratio MBC/MIC	Classification of extracts
		CMI	**CMB**		
Hydroéthanolic Maceration	$M_{Z.o}$	5000	10,000	2	Bactericidal
	$M_{V.p}$	625	2500	4	Bactericidal
	$M_{A.b}$	2500	5000	2	Bactericidal
Decoction	$D_{Z.o}$	10,000	N.d	/	/
	$D_{V.p}$	1250	5000	4	Bactericidal
	$D_{A.b}$	5000	10,000	2	Bactericidal
Rifampicin		3.91	3.91	1	Bactericidal

Legend: N.d = Not determined

The hydroethanolic extract of *V. paradoxa* had the best antimycobacterial activity on both strains. The results of the acute toxicity evaluation of this extract revealed that the single administration of the 2000 and 5000 mg/kg doses of this extract to healthy female rats caused no evidence of apparent toxicity after the first 4 hours. It had no effect on the behavior of the animals (mobility, appearance of the coat, aspect of the buttocks, eye colors, ...) in comparison with the control. In addition, no deaths were noted in the experimental groups after 14 days of study. This suggests that the lethal dose 50 (LD50) of this extract would be greater than 5000 mg/kg and that this latter would be classified according to the Globally Harmonized Classification System (GHS) to category 5 of low or no toxic substances [62]. These results are similar to those obtained by Mainasara *et al.* (2016a) [63] who also showed that the LD50 of the methanolic extract of *V. paradoxa* bark was greater than 5000 mg/kg. Figure 1 illustrates the effects of the hydroethanolic extract of *V. paradoxa* on the weight variation of the rats. It shows that animal body weights increased exponentially the first two days after treatment, then varied irregularly until the fourteenth day for all test groups and sensibly in a regular manner for the normal group. However, no significant difference in weight was observed between the animals treated with this extract at doses of 2000 and 5000 mg/kg and the control. In the same rank of idea, Mainasara *et al.* (2016b) [64] demonstrated by their work that the chronic administration of methanolic extract of *V. paradoxa* at doses up to 400 mg/kg for 30 days did not result in a significant variation in the weight of the rats during the period of treatment. The relative weight of the kidneys, liver, spleen, lungs and heart of the animals that received this extract after a 14-day follow-up is summarized in Figure 2. No significant difference ($p>0.05$) was observed between the relative weight of organs of animals treated at doses of 2000 and 5000 mg/kg of the hydroethanolic extract of *V. paradoxa* compared to control. Its effect on transaminase activity (AST and ALT) and creatinin concentration is shown in Table IV. No significant differences ($p>0.05$) in AST and ALT activities and creatinin concentration were observed between animals treated at 2000 and 5000 mg/kg of this extract compared to control.

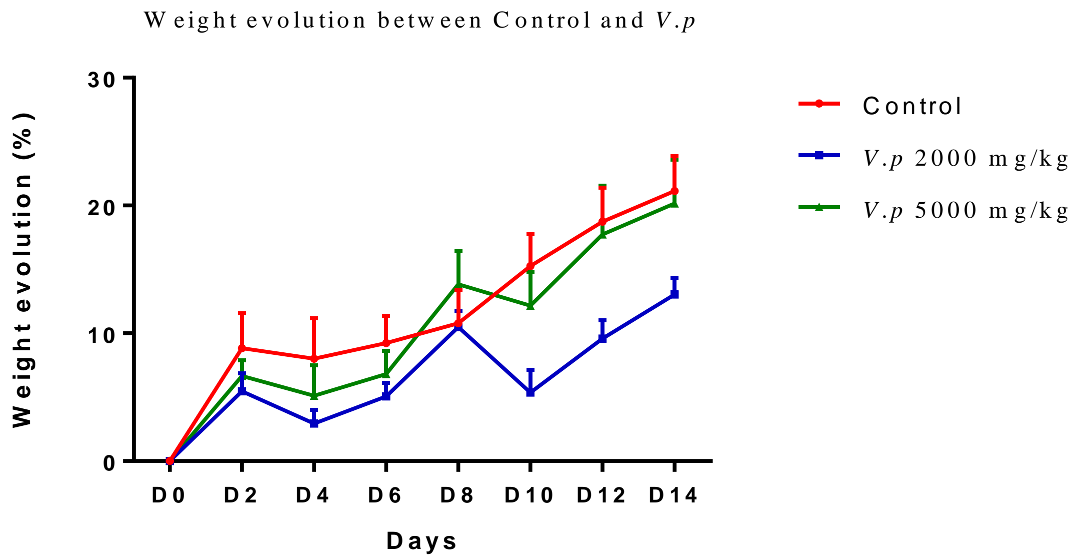

Figure 1 : Effects of hydroethanolic extract of *V. paradoxa* bark on the weight evolution of animals in acute toxicity

Each point represents the mean ± SEM ; n = 4; Control : rats treated with distilled water; *V. p* 2000 mg/kg and *V. p* 5000 mg/kg: rats treated with hydroethanolic extract of *V. paradoxa* respectively at doses of 2000 and 5000 mg/kg.

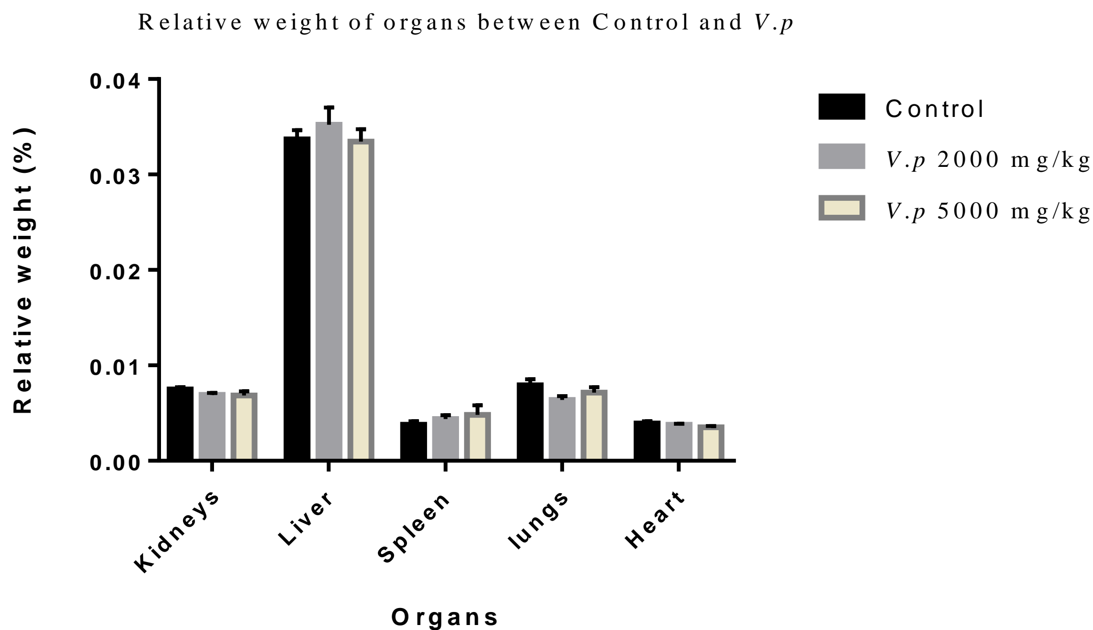

Figure 2 : Relative weight of animal organs that received hydroethanolic extract of *V. paradoxa* bark

Each bar represents the mean ± SEM ; n = 4; Control: rats treated with distilled water; *V. p* 2000 mg/kg and *V. p* 5000 mg/kg: rats treated with hydroethanolic extract of *V. paradoxa* respectively at doses of 2000 and 5000 mg/kg.

Table IV : Effects of hydroethanolic extract of *V. paradoxa* on transaminase activity (AST and ALT) and creatinin concentration

	Control	*V. p* 2000 mg/kg	*V. p* 5000 mg/kg
AST (UI/L)	4,481±0,397	5,471±0,600	3,977±0,563
ALT (UI/L)	4,263±0,689	5,878±0,333	4,190±0,563
Créatinin (µmol/L)	95,504±19,500	95,504±10,817	72,158±11,214

CONCLUSION

The results of this study highlights the importance to be given to plants, since they have many properties including antimycobacterial. The antimycobacterial potential and the non-toxicity of hydroethanolic extract (ethanol 30°) of the bark of *V. paradoxa* justifies the traditional use of this plant for the treatment of TB. However, given the fact that *M. tuberculosis* $H_{37}Rv$ was particularly sensitive to extracts of *V. paradoxa* and *A. boonei* (respectively strong and moderate activities), these plants are excellent candidates for the search for new molecules that can act against *M. tuberculosis* $H_{37}Rv$.

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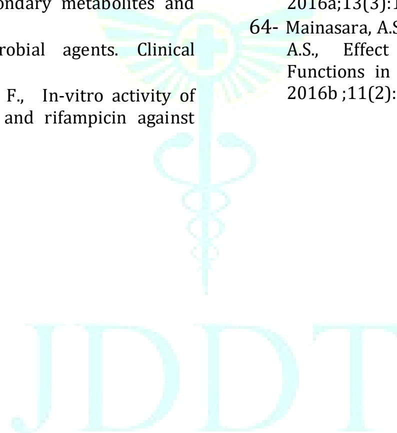

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