



## Review

## The search for plant activity against tuberculosis using breakpoints: A review



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## ABSTRACT

The present study proposes a discussion about the use of breakpoints when plant derivatives are used for investigating potential agents against *Mycobacterium tuberculosis* strains. A systematic review on these aspects was performed and supported that an arbitrary breakpoint may be considered inadequate in this kind of study. In addition, we propose that the adoption of this limiter should be done from the toxicity value found using the same plant derivative.

### 1. Introduction

Tuberculosis is a disease caused by mycobacteria belonging to the *Mycobacterium tuberculosis* complex (some changes in the taxonomy of mycobacteria were proposed by Gupta et al., 2018, but we chose to use a traditional taxonomy [1]) and is the leading cause of death by a single infectious agent worldwide, and it is estimated that around 10 million people have developed tuberculosis in 2017 [2]. One of its challenges is that only about 10% of the infected individuals evolve with the disease and, even though the remaining thrivingly contain the infection, the pathogen can persist in its latent form for many years, posteriorly causing the disease [3]. Even though the use of combined therapy for the last 2 decades reduced the occurrence of drug resistance [4], it remains the main challenge today for the treatment [2].

The development of strategies for control and eradication of tuberculosis has been the focus of the activities of the World Health Organization (WHO), defined mainly at the World Health Assembly in May 2014. In order to achieve the goal of reducing mortality and the incidence of tuberculosis, several action plans have been drawn, including the continuous search for new possible antimycobacterial drugs [2]. Due to the wide variety of natural products of plant origin with bioactive compounds, plants naturally consist of probable sources for the development of new antimycobacterial drugs [5].

To determine the minimum inhibitory concentration (MIC) of the

plant derivatives against *M. tuberculosis* strains, the most common method is performed by using microtiter plates and applying a substance that is able to detect the viability, such as resazurin or tetrazolium salts [5]. This method was initially implemented by Collins and Franzblau in 1997 [6] and allowed a fast and low-cost evaluation of mycobacterial growth under the action of different standardized antimicrobial agents or plant derivatives.

Due to the growing research on bioactive products recovered from plant derivatives and the development of adequate in vitro tests for screening of potential compounds, the recognition of some inherent flaws of this investigative process also emerged:

- (i). Difficulties to standardize the MIC tests of the studied plant compound, because plants respond to the variable intrinsic and extrinsic stimuli, altering their primary and secondary metabolism and consequently their components;
- (ii). Difficulties in interpreting the susceptibility test results of plant derivatives on mycobacterial strains as susceptible or resistant based on comparison with similar tests using standard antibiotic agents.

Regarding the first cited topic (i), it is coherent to understand that this is a permanent bias in this kind of studies, since it is definitely impossible to obtain the same plant compound, even if it is extracted

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from the same plant. Once the time interval between the collects of the plant material to carry out the new preparations of plant derivatives that will be submitted to new tests is always enough to allow that metabolic alterations occur in this individual. Thus, the search for antimycobacterial activities from a previously investigated species should not be discouraged, since the alterations of the natural products generated as a function of time can bring results that deny activities previously found for the plant species in question, or, may evidence the presence of antimycobacterial activity in materials previously tested and considered without antimycobacterial potential. In this context, the most frequently investigated plant species against mycobacteria of the *Mycobacterium tuberculosis* strains, as well as the MIC found in their results, should be identified in the literature.

The second topic (ii) leads to the need to carry out the literature review on breakpoints used as reference to the plant derivatives as a potential source of antimycobacterial drug in vitro assays against *M. tuberculosis*.

In order to clarify the second aspect mentioned above, a systematic review of the literature was developed based on studies that investigated plant derivatives as a potential source of antimycobacterial agent. The present work does not propose to modify the current concentrations of plant derivative solutions used during the assays, but to review and discuss the interpretation of the results of these tests.

## 2. Materials and methods

A systematic review of the literature was carried out through searches in the databases Google scholar ([scholar.google.com](http://scholar.google.com)), Scielo and Pubmed using the associations of the following words in Portuguese: antimycobacteriana; antimicrobiana e Mycobacterium; Mycobacterium e plantas. And in English: antimycobacterial; antimicrobial and Mycobacterium; Mycobacterium and plants. The search in the Google academic database also counted on the use of the terms: tuberculosis and MIC, in order to better direct presented results.

The obtained manuscripts were submitted to the second selection using the inclusion and exclusion criteria described below:

### 2.1. Inclusion criteria

- The antimicrobial susceptibility tests used microplates and viability indicators. Studies using the cited method and others together were not excluded;
- The mycobacteria submitted to the susceptibility tests must be the *Mycobacterium tuberculosis*, while others may be present in the same study, only the data referring to the mycobacteria belonging to the *M. tuberculosis* were considered;
- The materials tested against mycobacteria should be a plant derivative, such as: essential oils, organic extracts and fractions from extracts or from essential oils. Studies using purified substances were only discarded if they have not presented in the concomitant investigation of plant derivatives as the cited;
- Studies in English, Spanish or Portuguese;
- Investigations published between 1996 and 2015.

### 2.2. Exclusion criteria

#### 2.2.1. Review studies

- Studies published in non-indexed journals;
- Studies published in Events in the form of a summary;
- Studies published in languages other than English, Spanish or Portuguese;
- Studies that did not allow the free access to the full text;
- Studies that only present tests using isolated or synthetic substances;
- Studies appearing on more than one search path were considered only once.

**Table 1**

The applied checklist containing the mandatory information to keep the study as "included" and to collect the data from each study.

Checklist	
Tested plant species.	Insert the names of species
Plant derivatives used.	Insert the kind of plant derivative used
Minimum Inhibitory Concentrations (MIC) found in susceptibility assays.	Insert the values
MIC values considered as breakpoints.	Insert the values
Conclusion: The plant derivative was considered potentially active?	Yes or no

After the second selection, it was applied a third selection using the checklist described below to collect the data of the included studies. (Table 1).

## 3. Results and discussion

This review included 41 studies published between 1996 and 2015. The flow diagram (Fig. 1) below shows the sequence used to obtain the studies used and the respective data obtained through the application of our checklist described in Table 1.

As a result of the checklist application, we listed a total of 199 species of plants used for in vitro antimicrobial investigations against *M. tuberculosis*. Among the plant derivatives, 113 methanolic extracts, 39 hexane extracts, 27 extracts in dichloromethane, 32 extracts in ethyl acetate, 63 extracts in chloroform, 11 aqueous extracts and 5 essential oils were tested. The most prevalent plant parts used in the preparation of extracts were the aerial parts of plants (leaf, stem, bark and fruit). The most frequent species in the studies was *Byrsonima crassa*, present in 3 studies.

Of the 41 articles selected for the study, 23 used breakpoints in the research. Among these, only 1 studied considered breakpoint greater than 200 µg/mL (250 µg/mL). Eleven studies that presented breakpoints considered breakpoints values between 100 and 128 µg/mL. Five articles considered values equal to or less than 50 µg/mL. Additionally, 19 studies did not use breakpoints (Fig. 2 and Table 2).

In this sense, it is relevant to observe that the interpretations of the results varied arbitrarily. It should be noted that the concept of breakpoints used in antimicrobial susceptibility testing is based on previous studies considering a well-defined and long-tested protocol, such as EUCAST (European Committee on Antimicrobial Susceptibility Testing), CLSI (Clinical & Laboratory Standards Institute) or BrCAST (Brazilian Committee on Antimicrobial Susceptibility Testing). Despite that, it is mandatory to highlight that there is a continuous challenge relative to the breakpoint value when we take into account the mycobacteria from *Mycobacterium tuberculosis* Complex [48]. These protocols are able to inform the sensitivity profile of some specific bacteria to the standard antimicrobial agent tested, and it is done based on the breakpoint. On the other hand, when the studies use the same test to investigate about the potential of plant derivatives being a source of medicine against *M. tuberculosis*, the results, as was shown before, present that the plant derivative is a potential or not source of medicine also based in some breakpoint, which is based in other studies that do not investigate the same plant derivative. On this way, when an antimicrobial activity search is performed using plant derivative products, the MIC values identified by these studies may be considered as a measure that merits further reviews for future studies, since the inhibition of microbial growth has occurred. Also, this inhibition can inform the presence of active plant components against the tested microbial species, even if it is not a major component. This way, we propose the presentation of the results as information relative to the plant derivative's ability of inhibiting or not the mycobacterial growth. This change in the readout of the susceptibility tests should increase the

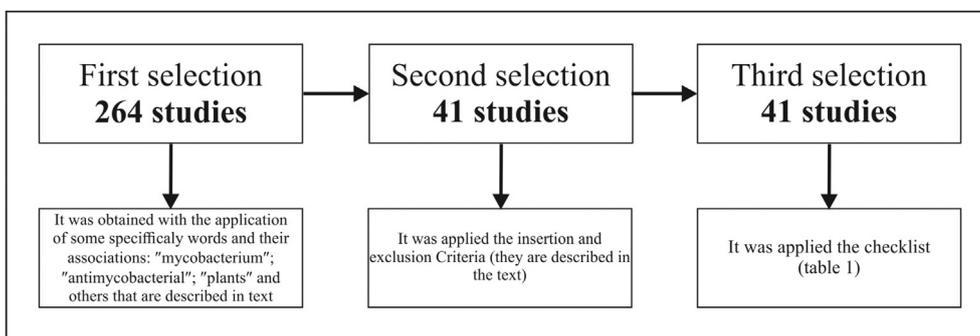


Fig. 1. Flow diagram represents the sequence of actions used to select the studies and to collect their respective data through the application of checklist described in Table 1.

number of plant derivatives as potential sources of medicines and, with the insertion of toxicity tests and new evaluations relative the chemical compounds of these ones, a better selection of plant derivatives could be done.

Regarding the definition of a breakpoint for a plant derivative to be considered active against *M. tuberculosis*, 19 studies did not report this value. Nevertheless, of these 19 studies, 8 considered as potentially active the plant derivatives tested, since they inhibited the mycobacterial growth. These studies showed MIC ranging from 2.5 µg/mL to 250 µg/mL.

Considering these results, it is clear that exists a lack of standardization of studies investigating the antimycobacterial activity using plant derivatives, since there is an absence of proposals for standardizing MIC tests for plant derivatives. As an example, the use of breakpoints to define a test material occurred in 55% of the studies analyzed, and, on the other hand, 19.5% of the studies (n = 8) concluded as potentially active the plant derivatives tested without definition of a breakpoint. In this sense, the authors of these studies (19.5%) consider that plant derivatives do not have a truth breakpoint,

and they may not have it since there is no a standardized method due to several variable aspects related to reproducibility, mixed compounds in extracts and other flaws. Taking into account the relevance of MIC values for the accomplishment of future studies for the development of therapeutic possibilities against *M. tuberculosis*, the identification of low MIC values generally increases the expectation that the tested plant derivatives will be effectively active, not only potential (Table 3).

Among the studies that used breakpoints, 10 had MIC values higher than the previously suggested breakpoints and were not considered as active potential (Table 4). This conclusion is doubtful to us since, even if the MIC found to be greater than the breakpoint considered by the study, the tested plant derivative should not be pulled out from the list of antimycobacterial potentials because there are still studies to be carried out on it, such as the purification and identification of the active component and its toxicity. In this sense, it would be more pertinent to consider the plant derivatives tested as capable of inhibiting growth and further studies on purified compounds should be performed.

In a study carried out by Machado et al. [49], in which they investigated the antimycobacterial activity of 11 plant derivatives, the

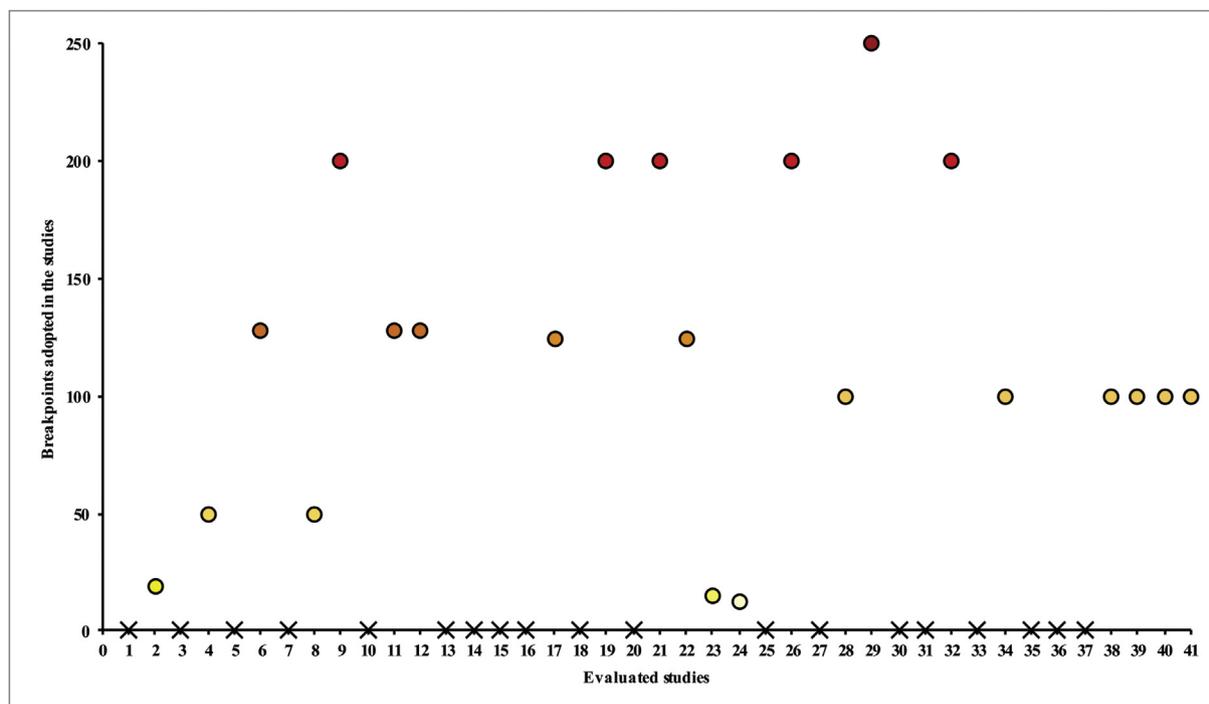


Fig. 2. Frequency of use of breakpoints in the studies and their respective values. Colored circles present colors varying gradually from the smallest to the highest breakpoint value. The "X" in the x-axis refers to the studies that did not use breakpoints in the investigations. The analyzed studies are listed in the x-axis of 1–41, and it is possible to consult them in the reference organized in Table 2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



Table 2 (continued)

#	Reference	Plant species	Plant derivatives	MIC found in assays	Breakpoint	Conclusion: active?	
3	Leitão et al., 2006 [9]	<i>Bauhinia microstachya</i> var. <i>massambabensis</i>	Ethanol extract of leaves	NF	NI	No	
			<i>Bauhinia microstachya</i> var. <i>microstachya</i>	Hexane extract of leaves		NF	No
		<i>Lippia origanoides</i>	Ethanol extract of leaves	NF		No	
			Dichloromethane extract of leaves	NF		No	
			Ethyl acetate extract of leaves	NF		No	
			N-butanolic extract of leaves	NF		No	
			Ethanol extract of leaves	NF		No	
			<i>Lippia alba</i> forma <i>intermedia</i>	Ethanol extract of leaves		NF	No
			<i>Lantana trifolia</i>	Dichloromethane extract of leaves		80 µg/mL	Yes
				Hexane extract of leaves		80 µg/mL	Yes
			<i>Hennecartia omphalandra</i>	Ethyl acetate extract of leaves		NF	No
				N-butanolic extract of leaves		NF	No
		Ethanol extract of stalk		NF		No	
		Hexane extract of stalk		NF		No	
		Dichloromethane extract of stalk		NF		No	
		Ethyl acetate extract of stalk		NF		No	
		N-butanolic extract of stalk		NF		No	
		<i>Vitex cooperi</i>		Ethanol extract of leaves		NF	No
				Ethanol extract of fruits		NF	No
		<i>Vitex cymosa</i>		Ethanol extract of bark		80 µg/mL	Yes
			Ethanol extract of wood	NF		No	
		<i>Vitex polygama</i>	Ethanol extract of fruits	NF		No	
			Hexane extract of leaves	NF		No	
		<i>Lippia integrifolia</i>	Hexane extract of fruits	NF		No	
			Dichloromethane extract of leaves	NF		No	
		<i>Lippia lacunosa</i>	Ethyl acetate extract of leaves	NF		No	
			Aqueous extract of aerial parts	NF		No	
			Methanol extract of aerial parts	NF		No	
			Dichloromethane extract of aerial parts	NF		No	
		<i>Lippia rotundifolia</i>	Ethanol extract of leaves	NF		No	
			Dichloromethane extract of leaves	25 µg/mL		Yes	
			Ethanol extract of leaves	NF		No	
Ethyl acetate extract of leaves	NF		No				
N-butanolic extract of leaves	NF		No				
Hexane extract of leaves	50 µg/mL		Yes				
4	Bertucci et al., 2009 [10]	<i>Eugenia masoni</i>	Acetone extract of leaves	200 µg/mL	50 µg/mL	No	
			Chloroform extract of leaves	200 µg/mL		No	
		<i>Eugenia repanda</i>	Acetone extract of leaves	100 µg/mL		No	
			Chloroform extract of leaves	100 µg/mL		No	
		<i>Myrcianthes cisplatensis</i>	Acetone extract of leaves	200 µg/mL		No	
			Chloroform extract of leaves	100 µg/mL		No	
		<i>Paullinia elegans</i>	Hydroethanol extract of leaves	> 200 µg/mL		No	
			Acetone extract of leaves	200 µg/mL		No	
			Chloroform extract of leaves	200 µg/mL		No	
			Hydroethanol extract of fruits	200 µg/mL		No	
		<i>Petunia sp</i>	Acetone extract of aereal parts	50 µg/mL		Yes	
			Chloroform extract of aereal parts	50 µg/mL		Yes	
5	Chavasco et al., 2014 [11]	<i>Ruprechtia laxiflora</i>	Hydroethanol extract of leaves	200 µg/mL	NI	No	
		<i>Bidens pilosa</i>	Hydroethanol extract of leaves, flowers, roots, stalk and fruits	NF		No	
		<i>Eugenia pyriformis</i>		NF			
		<i>Plinia cauliflora</i>		NF			
		<i>Heliconia rostrata</i>		NF			

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Table 2 (continued)

#	Reference	Plant species	Plant derivatives	MIC found in assays	Breakpoint	Conclusion: active?	
6	Pavan et al., 2009 [12]	<i>Anacardium humile</i>	Chloroform extract of leaves	2000 µg/mL	< 128 µg/mL	Yes	
			Methanol extract of leaves	500 µg/mL			
		<i>Mangifera indica</i>	Chloroform extract of leaves	4000 µg/mL			
			Methanol extract of leaves	2000 µg/mL			
			<i>Harcornia speciosa</i>	Chloroform extract of leaves			2000 µg/mL
				Methanol extract of leaves			4000 µg/mL
			<i>Ananas ananassoides</i>	Chloroform extract of leaves			2000 µg/mL
				Methanol extract of leaves			2000 µg/mL
			<i>Bromelia balansal</i>	Chloroform extract of fruits			2000 µg/mL
				Methanol extract of fruits			2000 µg/mL
			<i>Articun lappa</i>	Methanol extract of leaves			4000 µg/mL
			<i>Wilbrandia ebracteata</i>	Methanol extract of leaves			2000 µg/mL
			<i>Caesalpinia ferrea</i>	Methanol extract of leaves			4000 µg/mL
			<i>Curatella americana</i>	Chloroform extract of bark			62.5 µg/mL
				Methanol extract of bark			500 µg/mL
			<i>Davilla elliptica</i>	Chloroform extract of leaves			62.5 µg/mL
				Methanol extract of leaves			4000 µg/mL
			<i>Davilla nitida</i>	Chloroform extract of leaves			125 µg/mL
				Methanol extract of leaves			2000 µg/mL
			<i>Eriocaulon ligulatum</i>	Chloroform extract of scapes			1000 µg/mL
				Methanol extract of scapes			4000 µg/mL
			<i>Leiothrix flavescens</i>	Chloroform extract of scapes			125 µg/mL
				Methanol extract of scapes			2000 µg/mL
			<i>Syngonanthus arthrochichus</i>	Chloroform extract of pseudanthium			4000 µg/mL
				Chloroform extract of scapes			1000 µg/mL
			<i>Syngonanthus macrolepis</i>	Chloroform extract of scapes			1000 µg/mL
				Methanol extract of scapes			4000 µg/mL
			<i>Alchornea glandulosa</i>	Chloroform extract of leaves			4000 µg/mL
				Methanol extract of leaves			4000 µg/mL
			<i>Alchornea triplinervia</i>	Chloroform extract of leaves			4000 µg/mL
				Methanol extract of leaves			4000 µg/mL
			<i>Indigofera suffruticosa</i>	Chloroform extract of leaves			1000 µg/mL
				Methanol extract of leaves			125 µg/mL
			<i>Indigofera truxilensis</i>	Methanol extract of leaves			500 µg/mL
			<i>Strychnos pseudoquina</i>	Chloroform extract of leaves			125 µg/mL
				Methanol extract of leaves			4000 µg/mL
			<i>Byrsonima basiloba</i>	Chloroform extract of leaves			125 µg/mL
				Methanol extract of leaves			250 µg/mL
			<i>Byrsonima coccolobifolia</i>	Methanol extract of leaves			1000 µg/mL
			<i>Byrsonima crassa</i>	Chloroform extract of leaves			125 µg/mL
				Methanol extract of leaves			1000 µg/mL
				Chloroform extract of bark			2000 µg/mL
		Methanol extract of bark	1000 µg/mL				
	<i>Byrsonima fagifolia</i>	Chloroform extract of leaves	62.5 µg/mL				
		Methanol extract of leaves	500 µg/mL				
	<i>Byrsonima intermedia</i>	Chloroform extract of leaves	250 µg/mL				
		Methanol extract of leaves	2000 µg/mL				
	<i>Miconia cabuku</i>	Chloroform extract of leaves	250 µg/mL				
		Methanol extract of leaves	31.2 µg/mL				
	<i>Miconia rubiginosa</i>	Chloroform extract of leaves	250 µg/mL				
		Methanol extract of leaves	31.2 µg/mL				
	<i>Mouriri pusa</i>	Chloroform extract of leaves	4000 µg/mL				
		Methanol extract of leaves	2000 µg/mL				
	<i>Guapira noxia</i>	Chloroform extract of leaves	> 250 µg/mL				
		Methanol extract of leaves	31.2 µg/mL				
	<i>Neea theifera</i>	Chloroform extract of leaves	62.5 µg/mL				
		Methanol extract of leaves	250 µg/mL				
	<i>Quassia amara</i>	Chloroform extract of bark	250 µg/mL				
	<i>Cissus suscicaulis</i>	Chloroform extract of leaves	62.5 µg/mL				
	<i>Qualea grandiflora</i>	Chloroform extract of bark	62.5 µg/mL				
		Methanol extract of bark	1000 µg/mL				
	<i>Qualea multiflora</i>	Chloroform extract of bark	125 µg/mL				
		Methanol extract of bark	500 µg/mL				
7	Alvarado et al., 2011 [13]	<i>Drosera capillaris</i>	Methanol extract	1,25 µg/mL	NI	Yes	
				2.5 - 5 µg/mL			
8	León-Díaz et al., 2010 [14]	<i>Aristolochia taliscana</i>	Hexane extract of aerial parts	50 µg/mL	50 µg/mL	Yes	
9	Moreira et al., 2013 [15]	<i>Paepalanthus spp</i>	Ethanol extract of aerial parts	500 µg/mL	< 200 µg/mL	No	
10	Lopes et al., 2007 [16]	<i>Davilla elliptica</i> St. Hill	Chloroform extract of leaves	62.5 µg/mL	NI	Yes	
11	Cardoso et al., 2013 [17]	<i>Serjania erecta</i> Radlk	Ethanol extract of leaves	128 µg/mL	< 128 µg/mL	Yes	
			Ethanol extract of roots	256 µg/mL		No	

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Table 2 (continued)

#	Reference	Plant species	Plant derivatives	MIC found in assays	Breakpoint	Conclusion: active?			
12	Salazar et al., 2007 [18]	<i>Virola sp.</i>	Ethanol extract of leaves	48.64 µg/mL	~ 128 µg/mL	No			
			<i>Virola peruviana</i>	Ethanol extract of leaves		39.68 µg/mL	No		
			<i>Virola calophylla</i>	Ethanol extract of stalk		37.12 µg/mL	No		
		<i>Virola flexuosa</i>	Ethanol extract of leaves	113.92 µg/mL		No			
			Ethanol extract of leaves	121.6 µg/mL		Yes			
			Hexane extract of leaves	120.32 µg/mL		Yes			
		<i>Piper sp.</i>	Ethanol extract of leaves	117.76 µg/mL		Yes			
			<i>Piper peltatum</i>	Ethanol extract of leaves		44.8 µg/mL	No		
			<i>Piper hispidium</i>	Ethanol extract of leaves		34.56 µg/mL	No		
		<i>Piper auriculatum</i>	Ethanol extract of wood	121.6 µg/mL		Yes			
			Ethanol extract of leaves	34.56 µg/mL		No			
			13	Jiménez-Arellanes et al., 2013 [19]		<i>Moussonia deppeana</i>	Hexane extract of leaves	> 50 µg/mL	NI
Acetone extract of leaves	12.5 µg/mL	Yes							
Ethanol extract of leaves	> 50 µg/mL	No							
14	Camacho-Corona et al., 2008 [20]	<i>Citrus aurantifolia</i>			Hexane extract of peel	200 µg/mL	NI	No	
					Chloroform extract of bark	> 200 µg/mL		No	
					Methanol extract of bark	> 200 µg/mL		No	
		<i>Citrus sinensis</i>			Aqueous extract of bark	> 200 µg/mL		No	
					Hexane extract of peel	200 µg/mL		No	
					Chloroform extract of bark	> 200 µg/mL		No	
		<i>Foeniculum vulgare</i>			Methanol extract of bark	> 200 µg/mL		No	
					Aqueous extract of bark	> 200 µg/mL		No	
					Hexane extract of aerial parts	200 µg/mL		No	
		<i>Chloroform extract of aerial parts</i>	Chloroform extract of aerial parts	200 µg/mL	No				
			Methanol extract of aerial parts	> 200 µg/mL	No				
			Aqueous extract of aerial parts	> 200 µg/mL	No				
<i>Laurea tridentata</i>	Hexane extract of aerial parts	> 200 µg/mL	No						
	Chloroform extract of aerial parts	200 µg/mL	No						
	Methanol extract of aerial parts	> 200 µg/mL	No						
<i>Mentha pulegium</i>	Aqueous extract of aerial parts	> 200 µg/mL	No						
	Hexane extract of leaves	> 200 µg/mL	No						
	Chloroform extract of leaves	> 200 µg/mL	No						
<i>Musa acuminata</i>	Methanol extract of leaves	> 200 µg/mL	No						
	Aqueous extract of leaves	> 200 µg/mL	No						
	Hexane extract of stem	> 200 µg/mL	No						
<i>Nasturtium officinale</i>	Chloroform extract of stem	> 200 µg/mL	No						
	Methanol extract of stem	200 µg/mL	No						
	Aqueous extract of stem	> 200 µg/mL	No						
<i>Olea europaea</i>	Hexane extract of aerial parts	> 200 µg/mL	No						
	Chloroform extract of aerial parts	100 µg/mL	Yes						
	Methanol extract of aerial parts	> 200 µg/mL	No						
<i>Rosa centifolia</i>	Aqueous extract of aerial parts	> 200 µg/mL	No						
	Hexane extract of leaves	200 µg/mL	No						
	Chloroform extract of leaves	> 200 µg/mL	No						
<i>Methanol extract of leaves</i>	Methanol extract of leaves	> 200 µg/mL	No						
	Aqueous extract of leaves	> 200 µg/mL	No						
	Hexane extract of petals	> 200 µg/mL	No						
<i>Chloroform extract of petals</i>	Chloroform extract of petals	> 200 µg/mL	No						
	Methanol extract of petals	> 200 µg/mL	No						
	Aqueous extract of petals	> 200 µg/mL	No						
15	Hussain et al., 2008 [21]	<i>Piper sarmentosum</i>	Ethereal extract of leaves	25 µg/mL	NI	NI			
			Chloroform extract of leaves	25 µg/mL		NI			
			Methanol extract of leaves	12.5 µg/mL		Yes			
16	Navarro-García et al., 2011 [22]	<i>Aristolochia brevipes</i>	Dichloromethane extract of roots	12.5–25 µg/mL	NI	Yes			
			17	Leite et al., 2008 [23]		<i>Byrsonima crassa</i>	Chloroform extract of leaves	62.5 µg/mL	< 125 µg/mL
Methanol extract of leaves	1000 µg/mL	No							
18	Silva et al., 2009 [24]	<i>Piper cubeba</i>	Ethanol extract of seeds	62.5 µg/mL	NI	NI			
19	Suksamrarn et al., 2003 [25]	<i>Garcinia mangostana</i>	Methanol extract of fruits	6.25 µg/mL	< 200 µg/mL	Yes			
20	Hiebert et al., 2012 [26]	<i>Bulnesia sarmientoi</i>	Ethanol extract of bark	50 µg/mL	NI	Yes			

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Table 2 (continued)

#	Reference	Plant species	Plant derivatives	MIC found in assays	Breakpoint	Conclusion: active?
21	Askun et al., 2012 [27]	<i>Origanum acutidens</i>	Chloroform extract of aerial parts	12.5 µg/mL	6.25–200 µg/mL	Yes
			Ethyl acetate extract of aerial parts	6.3 µg/mL		Yes
			Methanol extract of aerial parts	25 µg/mL		Yes
		<i>Origanum sipyleum</i>	Chloroform extract of aerial parts	25 µg/mL		Yes
			Ethyl acetate extract of aerial parts	25 µg/mL		Yes
			Methanol extract of aerial parts	25 µg/mL		Yes
		<i>Salvia viridis</i>	Chloroform extract of aerial parts	12.5 µg/mL		Yes
			Ethyl acetate extract of aerial parts	12.5 µg/mL		Yes
			Methanol extract of aerial parts	25 µg/mL		Yes
		<i>Salvia microstegia</i>	Chloroform extract of aerial parts	12.5 µg/mL		Yes
			Ethyl acetate extract of aerial parts	25 µg/mL		Yes
			Methanol extract of aerial parts	12.5 µg/mL		Yes
		<i>Satureja boissieri</i>	Chloroform extract of aerial parts	0.4 µg/mL		No
			Ethyl acetate extract of aerial parts	12.5 µg/mL		Yes
			Methanol extract of aerial parts	12.5 µg/mL		Yes
		<i>Stachys byzantine</i>	Chloroform extract of aerial parts	25 µg/mL		Yes
			Ethyl acetate extract of aerial parts	12.5 µg/mL		Yes
			Methanol extract of aerial parts	25 µg/mL		Yes
		<i>Stachys cretica</i>	Chloroform extract of aerial parts	6.3 µg/mL		Yes
			Ethyl acetate extract of aerial parts	6.3 µg/mL		Yes
			Methanol extract of aerial parts	12.5 µg/mL		Yes
		<i>Stachys cretica smyrnaea</i>	Chloroform extract of aerial parts	12.5 µg/mL		Yes
			Ethyl acetate extract of aerial parts	12.5 µg/mL		Yes
			Methanol extract of aerial parts	12.5 µg/mL		Yes
<i>Thymus syriacus</i>	Chloroform extract of aerial parts	6.3 µg/mL	Yes			
	Ethyl acetate extract of aerial parts	3.1 µg/mL	No			
	Methanol extract of aerial parts	50 µg/mL	Yes			
<i>Thymus cilicicus</i>	Chloroform extract of aerial parts	6.3 µg/mL	Yes			
	Ethyl acetate extract of aerial parts	3.1 µg/mL	No			
	Methanol extract of aerial parts	12.5 µg/mL	Yes			

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Table 2 (continued)

#	Reference	Plant species	Plant derivatives	MIC found in assays	Breakpoint	Conclusion: active?				
22	Luo et al., 2011 [28]	<i>Adansonia digitata</i>	N-hexane extract of bark	NI	125 µg/mL	NI				
			Dichloromethane extract of bark	NI		NI				
			Ethyl acetate extract of bark	NI		NI				
		<i>Anacardium occidentale</i>	Ethanol extract of bark	NI		NI				
			N-hexane extract of roots	NI		NI				
			Dichloromethane extract of roots	NI		NI				
		<i>Ansellia africana</i>	Ethyl acetate extract of roots	NI		NI				
			Ethanol extract of roots	NI		NI				
			N-hexane extract of plant	NI		NI				
		<i>Artabotrys brachypetalus</i>	Dichloromethane extract of plant	NI		NI				
			Ethyl acetate extract of plant	NI		NI				
			Ethanol extract of plant	NI		NI				
		<i>Capparis tomentosa</i>	N-hexane extract of roots	NI		NI				
			Dichloromethane extract of roots	NI		NI				
			Ethyl acetate extract of roots	NI		NI				
		<i>Clerodendrum glabrum</i>	Ethanol extract of roots	NI		NI				
			N-hexane extract of leaves	NI		NI				
			Dichloromethane extract of leaves	NI		NI				
		<i>Combretum zeyheri</i>	Ethyl acetate extract of leaves	NI		NI				
			N-hexane extract of bark	NI		NI				
			Dichloromethane extract of bark	NI		NI				
		<i>Maerua edulis</i>	Ethyl acetate extract of bark	NI		NI				
			Ethanol extract of roots	NI		NI				
			N-hexane extract of roots	250 µg/mL		No				
		<i>Maerua juncea</i>	Dichloromethane extract of roots	NI		NI				
			Ethyl acetate extract of roots	NI		NI				
			Ethanol extract of roots	NI		NI				
		<i>Opuntia spp</i>	N-hexane extract of plant	NI		NI				
			Dichloromethane extract of plant	NI		NI				
			Ethyl acetate extract of plant	NI		NI				
		<i>Sarcostemma viminale</i>	Ethanol extract of plant	NI		NI				
			N-hexane extract of roots	NI		NI				
			Dichloromethane extract of roots	NI		NI				
		<i>Securidaca longepedunculata</i>	Ethyl acetate extract of roots	NI		NI				
			Ethanol extract of roots	NI		NI				
			N-hexane extract of roots	125 µg/mL		Yes				
		<i>Tabernaemontana elegans</i>	Dichloromethane extract of roots	NI		NI				
			Ethyl acetate extract of roots	NI		NI				
			Ethanol extract of roots	NI		NI				
		<i>Vernonia colorata</i>	N-hexane extract of leaves	NI		NI				
			Dichloromethane extract of leaves	NI		NI				
			Ethyl acetate extract of leaves	NI		NI				
23	Higuchi, 2007 [29]	<i>Byrsonima fagifolia</i>	Ethanol extract of leaves	NI	15.6 µg/mL	NI				
			Fraction of hexane extract of leaves	31.25 µg/mL		No				
			Fraction of methanol extract of leaves	7.81 µg/mL		Yes				
		<i>Byrsonima basiloba</i>	Fraction of dichloromethane extract of leaves	1.95 µg/mL		Yes				
			Fraction of methanol extract of leaves	31.25 µg/mL		No				
			Fraction of dichloromethane extract of leaves	7.81 µg/mL		Yes				
		<i>Byrsonima crassa</i>	Fraction of hexane extract of leaves	62.5 µg/mL		No				
			Fraction of methanol extract of leaves	7.81 µg/mL		Yes				
			Fraction of dichloromethane extract of leaves	250 µg/mL		No				
		<i>Byrsonima intermedia</i>	Fraction of hexane extract of leaves	500 µg/mL		No				
			24	Andrade-Ochoa et al., 2013 [30]		<i>Cuminum cyminum</i>	Essential oil of plant	12.5 µg/mL	12.5 µg/mL	Yes
						<i>Eugenia caryophyllata</i>	Essential oil of plant	25 µg/mL	Yes	
<i>Cinnamomum verum</i>	Essential oil of plant	12.5 µg/mL			Yes					
<i>Laurus nobilis</i>	Essential oil of plant	100 µg/mL			No					
<i>Pimpinella anisum</i>	Essential oil of plant	100 µg/mL			No					

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Table 2 (continued)

#	Reference	Plant species	Plant derivatives	MIC found in assays	Breakpoint	Conclusion: active?		
25	Balcha et al., 2014 [31]	<i>Allium ursinum</i>	Ethanol extract of bulb	250 µg/mL	NI	Yes		
		<i>Anethum graveolens</i>	Ethanol extract of aerial parts	NI		No		
		<i>Buddleja polystachya</i>	Ethanol extract of leaves	NI		No		
		<i>Croton macrostachyus</i>	Ethanol extract of leaves	NI		No		
		<i>Dodonaea angustifolia</i>	Ethanol extract of leaves	12.5 µg/mL		Yes		
26	Robles-Zepeda et al., 2013 [32]	<i>Pterolobium stellatum</i>	Ethanol extract of leaves	250 µg/mL	≤ 200 µg/mL	Yes		
		<i>Ambrosia confertiflora</i>	Methanol extract of leaves	200 µg/mL		Yes		
			Methanol extract of aerial parts	200 µg/mL		Yes		
		<i>Ambrosia ambrosioides</i>	Methanol extract of leaves	790 µg/mL		Yes		
			Methanol extract of roots	790 µg/mL		Yes		
		<i>Guaiacum coulteri</i>	Methanol extract of fruits	NI		No		
			Methanol extract of flowers	1000 µg/mL		No		
		<i>Acalypha californica</i>	Methanol extract of aerial parts	NI		No		
		<i>Schinus molle</i>	Methanol extract of aerial parts	NI		No		
		<i>Vallesia glabra</i>	Methanol extract of fruits	NI		No		
			Methanol extract of leaves	NI		No		
		<i>Baccharis salicifolia</i>	Methanol extract of aerial parts	NI		No		
		<i>Phoradenrom californicum</i>	Methanol extract of aerial parts	NI		No		
			Methanol extract of leaves	NI		No		
			Methanol extract of cortex	NI		No		
			<i>Acacia farnesiana</i>	Methanol extract of gum		NI	No	
				Methanol extract of flowers		NI	No	
		Methanol extract of seeds	NI	No				
		Methanol extract of leaves	NI	No				
		Methanol extract of cortex	NI	No				
		Methanol extract of roots	NI	No				
27	Gemechu et al., 2013 [33]	<i>Ocimum basilicum</i>	Methanol extract of seeds	25–100 µg/mL	NI	NI		
		<i>Croton macrostachyus</i>	Methanol extract of leaves	12.5–100 µg/mL		NI		
		<i>Calpurnia aurea</i>	Methanol extract of roots	25–100 µg/mL		NI		
		<i>Eucalyptus camaldulensis</i>	Methanol extract of leaves	6.25–50 µg/mL		NI		
		<i>Artemisia abyssinica</i>	Methanol extract of leaves	6.25–50 µg/mL		NI		
28	Lawal et al., 2011 [34]	<i>Uvaria afzelli</i>	Chloroform extract of bark	87.5 µg/mL	< 100 µg/mL	Yes		
		<i>Tetracera alnifolia</i>	Hexane extract of leaves	> 100 µg/mL		No		
			Chloroform extract of leaves	> 100 µg/mL		No		
			Hexane extract of bark	93.3 µg/mL		Yes		
29	Araujo et al., 2014 [35]	<i>Annona sylvatica</i>	Chloroform extract of bark	96.5 µg/mL	< 250 µg/mL	Yes		
			Methanol extract of leaves	> 250 µg/mL		NI		
			Ethyl acetate extract of leaves	115.2 µg/mL		NI		
30	Deng et al., 2008 [36]	<i>Angelica sinensis</i>	Hydromethanol extract	> 250 µg/mL	NI	NI		
			Chloroform extract of roots	63.1 µg/mL		NI		
			Methanol extract of roots	85.1 µg/mL		NI		
			Butanolic extract of roots	> 128 µg/mL		NI		
			Lyophilized extract of roots	64 µg/mL		NI		
			Aqueous extract of roots	> 128 µg/mL		NI		
			Bark extract of intern stem	NI		NI		
31	Inui et al., 2012 [37]	<i>Oplopanax horridus</i>	Dichloromethane extract of leaves	6.25–200 µg/mL	< 200 µg/mL	NI		
		32	Martins et al., 2013 [38]	<i>Duroia macrophylla</i>		Methanol extract of leaves	> 200 µg/mL	NI
						Dichloromethane extract of aerial parts	25–100 µg/mL	NI
						Methanol extract of aerial parts	> 200 µg/mL	NI
33	Molina-Salinas et al., 2011 [39]	<i>Leucophyllum frutescens</i>	Methanol extract of bark	NI	NI	NI		
34	Askun et al., 2013 [40]	<i>Stachys stmolea</i>	Fraction of petroleum ether	> 6400 µg/mL	< 100 µg/mL	NI		
			Fraction of ethyl acetate	> 6400 µg/mL				
			Fraction of methanol	> 6400 µg/mL				
		<i>Stachys thirkei</i>	Fraction of petroleum ether	> 6400 µg/mL				
			Fraction of ethyl acetate extract	> 6400 µg/mL				
			Fraction of methanol	> 6400 µg/mL				
		<i>Ballota acetabulosa</i>	Fraction of petroleum ether	200 µg/mL				
			Fraction of ethyl acetate	> 6400 µg/mL				
			Fraction of methanol	> 6400 µg/mL				
		<i>Thymus sibthorpii</i>	Fraction of petroleum ether	12.5 µg/mL				
			Fraction of ethyl acetate	12.5 µg/mL				
			Fraction of methanol	800 µg/mL				
		<i>Satureja aintabensis</i>	Fraction of petroleum ether	25 µg/mL				
			Fraction of ethyl acetate	12.5 µg/mL				
			Fraction of methanol	100 µg/mL				
		<i>Micromeria juliana</i>	Fraction of petroleum ether	1600 µg/mL				
			Fraction of ethyl acetate	100 µg/mL				
	Fraction of methanol	1600 µg/mL						
35	Green et al., 2011 [41]	<i>Bridelia micrantha</i>	N-hexane sub-fraction of ethyl acetate fractions from acetone extracts of bark	8.25 µg/mL	NI	NI		
36	Ma et al., 2005 [42]	<i>Micromelum hirsutum</i>	Dichloromethane extract of bark	12.5 µg/mL	NI	NI		

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Table 2 (continued)

#	Reference	Plant species	Plant derivatives	MIC found in assays	Breakpoint	Conclusion: active?
37	Barrows et al., 2007 [43]	<i>Evodia elleryana</i>	Hexane extract of stalk, bark and leaves Ethyl acetate extract of stalk, bark and leaves Methanol extract of stalk, bark and leaves	NI NI NI	NI NI NI	Yes
38	Jiménez-Arellanes et al., 2012 [44]	<i>Persea americana</i>	Chloroform extract of seeds Ethanol extract of seeds	50 µg/mL > 100 µg/mL	< 100 µg/mL	NI
39	Nielsen et al., 2012 [45]	<i>Acacia karroo</i> <i>Erythrophleum lasianthum</i> <i>Knowltonia vesicatoria</i> <i>Ptaeroxylon obliquum</i> <i>Salvia africana-lutea</i>	Methanol extract of stalk Methanol extract of stalk Methanol extract of aerial parts Methanol extract of bark Methanol extract of aerial parts	2500 µg/mL 625 µg/mL 39.06 µg/mL 156.25 µg/mL 312.50 µg/mL	< 100 µg/mL	NI
40	Fouotsa et al., 2013 [46]	<i>Garcinia nobilis</i>  <i>Orcia suaveolens</i>	Methanol extract of bark Dichloromethane extract of bark Methanol extract of wood Dichloromethane extract of wood Methanol extract of roots	128 µg/mL 256 µg/mL 256 µg/mL 512 µg/mL 512 µg/mL	< 100 µg/mL	No
41	Kuete et al., 2012 [47]	<i>Dioscorea bulbifera</i>	Methanol extract of bulb Fraction of ethyl acetate Sub-fraction of ethyl acetate	64–256 µg/mL 32–256 µg/mL 16–64 µg/mL	< 100 µg/mL	Yes

Notes: NF – Not Found; NI – Not Informed.

Table 3

List of plant derivatives with their respective antimycobacterial activities obtained from 16 studies that showed a MIC ≤ 20 µg/mL.

Plant species	Activity extract	MIC (µg/mL)	Reference	#
<i>Casearia sylvestris</i>	Ethanol extract of leaves	3.12	Ramos et al., 2008 [8]	2
<i>Peschiera affinis</i>	Ethanol extract of leaves	≤ 0.20		
<i>Plathymenia foliolosa</i>	Ethanol extract of leaves	0.78		
<i>Pouteria filipes</i>	Ethanol extract of leaves	0.78		
<i>Psychotria vellosiana</i>	Ethanol extract of leaves	≤ 0.20		
<i>Drosera capillaris</i>	Methanol extract of plant	1.25	Alvarado et al., 2011 [13]	7
<i>Drosera capillaris</i>	Methanol extract of plant	2.5–5		
<i>Moussonia deppeana</i>	Acetone extract of leaves	12.5	Jiménez-Arellanes et al., 2013 [19]	13
<i>Piper sarmentosum</i>	Methanol extract of leaves	12.5	Hussain et al., 2008 [21]	15
<i>Aristolochia brevipes</i>	Dichloromethane extract of roots	12.5–25	Navarro-García et al., 2011 [22]	16
<i>Garcinia mangostana</i>	Methanol extract of fruits	6.25	Suksamrarn et al., 2003 [23]	19
<i>Origanum acutidens</i>	Chloroform extract of aerial parts	12.5	Askun et al., 2012 [27]	21
<i>Origanum acutidens</i>	Ethyl acetate extract of aerial parts	6.3		
<i>Salvia viridis</i>	Chloroform extract of aerial parts	12.5		
<i>Salvia viridis</i>	Ethyl acetate extract of aerial parts	12.5		
<i>Salvia microstegia</i>	Chloroform extract of aerial parts	12.5		
<i>Salvia microstegia</i>	Methanol extract of aerial parts	12.5		
<i>Satureja boissieri</i>	Chloroform extract of aerial parts	0.4		
<i>Satureja boissieri</i>	Ethyl acetate extract of aerial parts	12.5		
<i>Satureja boissieri</i>	Methanol extract of aerial parts	12.5		
<i>Stachys byzantine</i>	Extract ethyl acetate of aerial parts	12.5		
<i>Stachys cretica</i>	Chloroform extract of aerial parts	6.3		
<i>Stachys cretica</i>	Ethyl acetate extract of aerial parts	6.3		
<i>Stachys cretica</i>	Methanol extract of aerial parts	12.5		
<i>Stachys cretica smyrnaea</i>	Chloroform extract of aerial parts	12.5		
<i>Stachys cretica smyrnaea</i>	Ethyl acetate extract of aerial parts	12.5		
<i>Stachys cretica smyrnaea</i>	Methanol extract of aerial parts	12.5		
<i>Thymus syriacus</i>	Chloroform extract of aerial parts	6.3		
<i>Thymus syriacus</i>	Ethyl acetate extract of aerial parts	3.1		
<i>Thymus cilicicus</i>	Chloroform extract of aerial parts	6.3		
<i>Thymus cilicicus</i>	Ethyl acetate extract of aerial parts	3.1		
<i>Thymus cilicicus</i>	Methanol extract of aerial parts	12.5		
<i>Tabernaemontana elegans</i>	Ethyl acetate extract of roots	15.6	Luo et al., 2011 [28]	22
<i>Byrsonima fagifolia</i>	Fraction of methanol extract of leaves	7.81	Higuchi, 2007 [29]	23
<i>Byrsonima fagifolia</i>	Fraction of dichloromethane extract of leaves	1.95		
<i>Byrsonima basiloba</i>	Fraction of dichloromethane extract of leaves	7.81		
<i>Byrsonima crassa</i>	Fraction of methanolic extract of leaves	7.81		
<i>Cuminum cymimum</i>	Essential oil of plant	12.5	Andrade-Ochoa et al., 2013 [30]	24
<i>Cinnamomum verum</i>	Essential oil of plant	12.5		
<i>Dodonaea angustifolia</i>	Ethanol extract of leaves	12.5	Balcha et al., 2014 [31]	25
<i>Duroia macrophylla</i>	Dichloromethane extract of leaves	6.25–200	Martins et al., 2013 [38]	32
<i>Thymus sibthorpii</i>	Fraction of petroleum ether	12.5	Askun et al., 2013 [40]	34
<i>Thymus sibthorpii</i>	Fraction of ethyl acetate	12.5		
<i>Satureja aintabensis</i>	Fraction of ethyl acetate	12.5		
<i>Bridelia micrantha</i>	N-hexane sub-fraction of ethyl acetate fractions from acetone extracts of bark	8.25	Green et al., 2011 [41]	35
<i>Micromelum hirsutum</i>	Dichloromethane extract of bark	12.5	Ma et al., 2005 [42]	36
<i>Dioscorea bulbifera</i>	Sub-fraction of ethyl acetate	16–64	Kuete et al., 2012 [47]	41

**Table 4**

List of plant derivatives not considered potentially active in studies that presented MIC above the defined breakpoint.

Plant species	Activity extracts	Identified MIC in assays (µg/mL)	Breakpoint (µg/mL)	Reference	#
<i>Eugenia mansonii</i>	Acetone extract of leaves	200	50	Bertucci et al., 2009 [10]	4
<i>Eugenia mansonii</i>	Chloroform extract of leaves	200	50		4
<i>Eugenia repanda</i>	Acetone extract of leaves	100	50		4
<i>Eugenia repanda</i>	Chloroform extract of leaves	100	50		4
<i>Myrcianthes cisplatensis</i>	Acetone extract of leaves	200	50		4
<i>Myrcianthes cisplatensis</i>	Chloroform extract of leaves	100	50		4
<i>Paullinia elegans</i>	Hydroethanol extract of leaves	> 200	50		4
<i>Paullinia elegans</i>	Acetone extract of leaves	200	50		4
<i>Paullinia elegans</i>	Chloroform extract of leaves	200	50		4
<i>Paullinia elegans</i>	Hydroethanol extract of fruits	200	50		4
<i>Ruprechtia laxiflora</i>	Hydroethanol extract of leaves	200	50		4
<i>Paepalanthus spp</i>	Ethanol extract of aerial parts	500	< 200	Moreira et al., 2013 [15]	9
<i>Serjania erecta Radlk</i>	Ethanol extract of roots	256	< 128	Cardoso et al., 2013 [17]	11
<i>Byrsonima crassa</i>	Methanol extract of leaves	1000	< 125	Leite et al., 2008 [23]	17
<i>Maerua edulis</i>	Hexane extract of roots	250	125	Luo et al., 2011 [28]	22
<i>Byrsonima fagifolia</i>	Fraction of hexane extract of leaves	31.25	15.6	Higuchi, 2007 [29]	23
<i>Byrsonima basiloba</i>	Fraction of methanol extract of leaves	31.25	15.6		
<i>Byrsonima crassa</i>	Fraction of hexane extract of leaves	62.5	15.6		
<i>Byrsonima crassa</i>	Fraction of dichloromethane extract of leaves	250	15.6		
<i>Byrsonima intermedia</i>	Fraction of hexane extract of leaves	500	15.6		
<i>Laurus nobilis</i>	Essential oil of plant	100	12.5	Andrade-Ochoa et al., 2013 [30]	24
<i>Pimpinella anisum</i>	Essential oil of plant	100	12.5		
<i>Guaiaecum coulteri</i>	Methanol extract of flowers	1000	≤200	Robles-Zepeda et al., 2013 [32]	26
<i>Tetracera alnifolia</i>	Hexane extract of leaves	> 100	< 100	Lawal et al., 2011 [34]	28
<i>Tetracera alnifolia</i>	Chloroform extract of leaves	> 100	< 100		
<i>Garcinia nobilis</i>	Methanol extract of bark	128	< 100	Fouotsa et al., 2013 [46]	40
<i>Garcinia nobilis</i>	Dichloromethane extract of bark	256	< 100		
<i>Orcia suaveolens</i>	Methanol extract of wood	256	< 100		
<i>Orcia suaveolens</i>	Dichloromethane extract of wood	512	< 100		
<i>Orcia suaveolens</i>	Methanol extract of roots	512	< 100		

value of 200 µg/mL was assigned as breakpoint. The essential oil of fruits of *Pterodon emarginatus* (Vogel) was identified as promising against *M. tuberculosis*. In another study developed by Machado et al. [50], the essential oil used in the previous study [8] was used to investigate a potential tool to be used in antimicrobial susceptibility tests. The essential oil was tested against *M. bovis*, that in the previous study had a MIC greater than that one found against *M. tuberculosis* and also because this plant derivative had a proven gastroprotective action [51], a characteristic that could serve as an ally in a therapy that used this essential oil orally. In this study, concentrations of 625 µg/mL, 1500 µg/mL and 2500 µg/mL of the essential oil of the *P. emarginatus* fruits were used and, although the MIC found by the 96-well microplate method was 625 µg/mL, using the tool (interferometry), it was possible to detect mycobacterial growth after 13 days of culture, even at the concentration of 1500 µg/mL. On the other hand, this growth was not realized in the highest concentration tested, even after 30 days. In another study, Machado et al. [52] used the hexane extract of fruits from *P. emarginatus* against *M. bovis* to verify the use of a new tool in antimicrobial susceptibility tests. In this study, the results showed that the MIC value of the hexane extract identified by the same method used in the study published in 2015<sup>a</sup> was the same, despite using another plant sample. However, only using the interferometer as a tool, it was possible to verify that the ideal concentration to inhibit the growth of mycobacteria longer had to be larger than the MIC identified by the microplate method. Another relevant aspect of this narrative lies in the fact that both studies [9,11] used more than one research method to infer the antimycobacterial potential of the plant derivative tested, which corroborates our proposal of abandoning the adoption of breakpoints in studies with plant derivatives that seek to identify antimycobacterial potential, since in fact, this limit is not able to define the real potential activity of the plant derivatives tested against mycobacteria strains.

According to Tempone and co-workers (2011) [53], a limit of 300 µg/mL should be considered as a breakpoint to avoid problems of microscopic analysis and the colorimetric or fluorimetric viability tests.

Despite this, the colorimetric tests using resazurin or Alamar Blue did not show problems using higher concentration than 300 µg/mL [52]. Thus, even if we assume the risk of having problems in the readout of colorimetric or fluorimetric tests by accepting higher concentrations, the possibility of finding a source of medicine against tuberculosis should be considered over the risk of having this kind of problem, in which case, the worst that could happen would be considering it as a non potential source of medicine.

Besides the problem already mentioned, there is also the possibility of the vehicle concentration in the tested solution when it is higher than 300 µg/mL become a problem, which could lead to a false positive result, once the vehicle could be the inhibitor agent and not the active principle of the plant derivative tested. To solve this issue, we propose adopting the continued use of control group that evaluate the behavior of a solution of the vehicle testing it as an inhibitor agent.

#### 4. Final considerations and conclusion

The analysis on the use of breakpoints in the investigations that use plant derivatives in the search for potential antimycobacterial agents allowed to make the following considerations:

- There is no consensus among the studies about choosing breakpoints;
- The use of breakpoints in these investigations may generate false positive results if the MIC values found are lower than those of the breakpoint and, through other methods of antimicrobial susceptibility testing, mycobacterial growth is still detected;
- The use of breakpoints in these investigations may generate false negative results, since MIC values above the cutoff values may present a higher bactericidal action when verified through another susceptibility test method to antimicrobial agents;
- Consideration of a plant derivative as an antimycobacterial potential, may only use a breakpoint when this value is derived from previous research on the toxicity level of the tested material.

Thus, we propose that future studies that search for an antimycobacterial activity using plant derivatives, no longer make use of this limiter and show the results as “the plant derivative was able to inhibit or not”, or define its use from the previous identification of toxic concentrations of the tested material.

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## Declarations of interest

None.

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