

Screening antimycobacterial activity of *Baccharis dracunculifolia*, *Centella asiatica*, *Lantana camara* and *Pterodon emarginatus*

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RESUMO: Atividade anti-micobacteriana de *Baccharis dracunculifolia*, *Centella asiatica*, *Lantana camara* e *Pterodon emarginatus*. A investigação permanente de novas drogas antimicobacterianas é necessária no programa de erradicação da tuberculose e de outras doenças relacionadas com micobactérias. O objetivo deste estudo foi buscar novas fontes de drogas antimicobacterianas usando material vegetal. Neste estudo, 11 materiais de base vegetal (extratos, óleos essenciais e algumas frações) foram avaliados contra 5 espécies de micobactérias. Estes materiais foram obtidos a partir de 4 espécies de plantas medicinais tradicionalmente utilizadas como terapêutica geral para diferentes doenças e, especificamente, no tratamento de tuberculose (*Baccharis dracunculifolia*, *Centella asiatica*, *Lantana camara*, *Pterodon emarginatus*). Os ensaios foram realizados em microplacas com resazurina contra duas espécies do Complexo *Mycobacterium tuberculosis* e 3 espécies de micobactérias não tuberculosas. Os resultados mostraram o extrato hexânico e o óleo essencial de frutos de *P. emarginatus* como potenciais fontes para drogas antimicobacterianas contra quatro espécies de micobactérias testadas. A fração hexânica do extrato metanólico das folhas de *C. asiatica* também apresentou significativa inibição do crescimento de micobactérias apenas contra *M. chelonae*. Em conclusão, foi possível contribuir para as investigações de antimicobacterianos por apresentar três novas amostras de plantas com atividade antimicrobiana significativa contra quatro *Mycobacterium* spp e sugerir a realização de estudos futuros sobre as propriedades antimicobacterianas de frutos de *P. emarginatus*.

Palavras-chave: antimicrobiano, Complexo *Mycobacterium tuberculosis*, ensaio em microplacas com resazurina, micobactérias não-tuberculosas, vegetais.

ABSTRACT: The permanent investigation of new antimycobacterial drugs is necessary for the eradication programs of tuberculosis and other mycobacterium-related diseases. The aim of the present study is to search for new sources of antimycobacterial drugs using plant materials. In this study, 11 plant materials (extracts, essential oils and some fractions) obtained from 4 species of medicinal plants traditionally used as general therapeutics for different illnesses and specifically as treatment of tuberculosis, were evaluated using the microplate resazurin

assay against 2 species of the *Mycobacterium tuberculosis* Complex and 3 nontuberculous mycobacteria. The results showed the hexane extract and the essential oil from fruits of *Pterodon emarginatus* (Vogel) as potential sources of antimycobacterial drugs against 4 species of tested mycobacteria. The hexane fraction of methanol extract from leaves of *Centella asiatica* also presented significant mycobacterial growth inhibition, but against *M. chelonae* only. In conclusion, it was possible to contribute to the antimycobacterial investigations by presenting three new samples of plants with significant antimicrobial activity against four *Mycobacterium* spp and suggest future studies about the antimycobacterial properties of fruits from *P. emarginatus*.

Keywords: Antimicrobial, Mycobacterium tuberculosis Complex, Microplate Resazurin Assay, Nontuberculous mycobacteria, Plants.

INTRODUCTION

The *Mycobacterium* sp is a genus of the Mycobacteriaceae family. They are aerobic microorganisms found in different habitats and they are able to promote relevant diseases such as tuberculosis, mycobacteriosis and leprosy. These diseases are promoted by different species of *Mycobacterium* spp such as: *Mycobacterium tuberculosis* and *M. bovis* (some etiologic agents of tuberculosis) and the species *M. abscessus*, *M. chelonae* or *M. smegmatis* (some *Mycobacterium* spp responsible for mycobacteriosis) and *M. leprae*, the etiologic agent of leprosy. Tuberculosis and leprosy are contagious, and the treatment of these cited diseases takes much longer than treating other types of bacterial infections (6-12 months). Thus the possibility of failure is high and it contributes to the appearance of mutant drug-resistant strains. (Brown-Elliott et al., 2012; Delogo et al., 2013).

The World Health Organization recently published good news about the profile of tuberculosis and leprosy around the world, presenting a reduction of their incidence, but also the requirement of continuous financial support to pursue the eradication of these diseases (WHO 2014a, 2014b). On the other hand, relative to mycobacteriosis cases, the number of mycobacteriosis cases is still growing and most cases are related to immunosuppressive causes and surgical interventions using catheters (Brown-Elliott et al., 2012; Pitombo et al., 2009).

Many studies aiming at new alternatives for treatment for mycobacteriosis and tuberculosis are being performed using plant samples which are evaluated by *in vitro* tests of susceptibility to possible antimicrobial compounds (Bueno et al., 2011).

According to the literature, numerous investigations using plant samples have been undertaken with respect to their antimycobacterial activities, as can be observed for *Baccharis dracunculifolia* D.C. (Asteraceae); *Centella asiatica* (L.) Urb. (Apiaceae), *Lantana camara* L. (Verbenaceae) and *Pterodon emarginatus* V. (Fabaceae) (Da Silva Filho et al., 2008; Feresin et al., 2003; Ghisalberti, 2000; Herbert et al., 1994;

Kirimuhuzya et al., 2009; Machado et al., 2012a; Suresh et al., 2010; Verdi et al., 2005).

Baccharis dracunculifolia is a native plant of South America and is found in Argentina, Bolivia, Brazil and Uruguay. It is used by the bees to produce the green propolis, which has recognized antimicrobial and anti-inflammatory activities (Feresin et al., 2003; Verdi et al., 2005). The antimicrobial activity of *B. dracunculifolia* has been studied (Ferronato, 2007; Da Silva Filho et al., 2008), but the effect of its essential oil against *Mycobacterium* spp has not been investigated to our knowledge.

Lantana camara is a plant frequently used as an ornament in gardens and is also commonly known for its potentially high toxicity due to the presence of hepatotoxic triterpenes (Ghisalberti, 2000). In addition, this plant is widely used in Africa for the treatment of diseases such as tuberculosis. Previous studies revealed a relevant antimycobacterial activity of this plant against *M. tuberculosis* using the methanol extract from its leaves (Kirimuhuzya et al., 2009), and the absence of toxicity of its essential oil when it was administered to mice (Machado et al., 2012b).

Centella asiatica is a traditional plant used in Ayurvedic medicine. Numerous investigations have shown its biological properties such as its cytotoxic action against cancer cells, its anti-inflammatory activity, its potential use in treatment of neurological disorders, all found in the water extract from its leaves (Barbosa et al., 2008; Pittella et al., 2009). Currently, it is often related to cosmetics for weight reduction according to scientific evaluations presented by Primastuti et al. (2013). However, its previous medicinal folk use was associated with treatment of leprosy lesions in India (Herbert et al., 1994) and a recent study detected antimycobacterial activity in the ethanol extract of its leaves against *M. tuberculosis* through microplate assay (Suresh et al., 2010).

Pterodon emarginatus is a native Brazilian plant that has mainly been studied in relation

to the treatment of inflammatory processes caused by arthritis and other diseases (Dutra et al., 2009; Hoscheid et al., 2013). Regarding the antimycobacterial activity of this plant, few investigations have been carried out (Lima et al., 2006; Machado et al. 2012a), and due to the high variability of its secondary metabolites (Alves et al., 2013), new explorations must be realized.

This study aims to present novel potential sources of drugs for treating mycobacteriosis and tuberculosis. Therefore, the susceptibility of five mycobacteria species (*M. abscessus*, *M. chelonae*, *M. smegmatis*, *M. tuberculosis*, *M. bovis*) to 11 plant samples obtained from four plant species (*B. dracunculifolia*, *C. asiatica*, *L. camara*, *P. emarginatus*) was evaluated.

MATERIAL AND METHOD

The plant samples and *Mycobacterium* spp used are listed in Tables 1 and 2 respectively. All vouchers were deposited in the Herbarium Leopoldo Krieger from the Botany Department of the Federal University of Juiz de Fora (UFJF) – Minas Gerais – Brazil and they were identified by Dr. Fátima Regina Gonçalves Salimena (Herbarium Leopoldo Krieger from Botany Department of the Federal University

of Juiz de Fora (UFJF) – Minas Gerais – Brazil) (Tables 1 and 2).

Pterodon emarginatus

Dry fruits of *P. emarginatus* were collected in September 2006 in the district of Três Marias (Minas Gerais – Brazil) which belongs to the Cerrado biome (tropical Brazilian savanna).

The extracts were obtained according to the method described by Costa (2000). Fruits (30 g) were cut and subjected to hexane extraction during 24 hours in a Soxhlet extractor. The still pot was replaced in order to exchange solvents. The following solvents were used: ethyl acetate, butanol, and methanol. These solvents were removed by means of a rotary evaporator (R-114, Buchi–Swiss) to obtain respectively hexane (HEPE), ethyl acetate (EAEPE), butanol (BEPE) and methanol (MEPE) extracts.

Fruits were cut and essential oil was extracted in a Clevenger type apparatus during 4 hours. The essential oil (essential oil from *P. emarginatus* - EOPE) and MEPE were kept at -20°C in an amber flask.

Centella asiatica

Adult leaves of *C. asiatica*, grown under controlled farming on the campus of Federal

TABLE 1. Overview of evaluated plants.

Scientific Name (Family)	Part Used	Extract	Voucher	Brazilian Folk Name
<i>Pterodon emarginatus</i> Vogel (Fabaceae)	Fruits	Hexane Extract (HEPE)	CESJ 48077	Sucupira, sucupira branca, faveiro
		Ethyl acetate Extract (EAEPE)		
		Butanol Extract (BEPE)		
		Methanol Extract (MEPE)		
		Essential Oil (EOPE)		
<i>Centella asiatica</i> (L.) Urb. (Apiaceae)	Leaves	Aqueous Extract (AECA)	CESJ 24610	Centelha, centelha asiática
		Methanol Extract (MECA)		
		Hexane Fraction (HFCA)		
		Chloroform-Hexane Fraction (CHFCA)		
<i>Lantana camara</i> L. (Verbenaceae)	Leaves	Essential Oil (EOLC)	CESJ 46090	Cambará, chumbinho
<i>Baccharis dracunculifolia</i> D.C. (Asteraceae)	Aerial Parts	Essential Oil (EOBD)	CESJ 46087	Alecrim-do-campo

TABLE 2. Overview of evaluated mycobacteria.

Group	Species
<i>Mycobacterium tuberculosis</i> Complex	<i>Mycobacterium bovis</i> – BCG Moreau
	<i>Mycobacterium tuberculosis</i> – H37Rv – ATCC 27294
	<i>Mycobacterium smegmatis</i> – ATCC 14468
Non-tuberculous mycobacteria	<i>Mycobacterium abscessus</i> – ATCC 5752
	<i>Mycobacterium chelonae</i> – ATCC 199777

University of Juiz de Fora - Brazil (S21°46'46.4" W43°22'13.3" elevation 865 m), were collected and dried at room temperature. The aqueous extract of *C. asiatica* (AECA) was prepared adding 200 g of chopped dried material in 4 L of water during 24 h without heating, as described in previous studies (Pittella et al., 2009; Primastuti et al., 2013). The final solution was filtered and freeze-dried, a process also known as lyophilization, (Thermo – Thermo Electron, USA) and kept at -80 °C in an amber flask. The methanol extract of *C. asiatica* (MECA) was obtained using 200 g of dried material exposed to methanol (1900 mL) during 2 h in a Soxhlet extractor. The extract obtained was filtered, concentrated and kept at -80 °C in an amber flask. The fractions were obtained using 10 g of MECA first diluted with 100mL of ethanol (10%). This solution was mixed with 100 mL of hexane in a separatory funnel. The aqueous phase and the hexane phase were then collected after three additional times. Then 100 mL of the aqueous phase was mixed with chloroform-hexane (2:8), so the chloroform-hexane phase was obtained. In this way, the hexane fraction (HFCA) obtained from MECA and the chloroform-hexane fraction (CHFCA), also obtained from MECA, were extracted respectively from the concentration of the hexane phase and the chloroform-hexane phase and afterwards kept at -80 °C in an amber flask.

Lantana camara and Baccharis dracunculifolia

Leaves of *L. camara* (900 g) and aerial parts of *B. dracunculifolia* (900 g) were collected in Juiz de Fora - Minas Gerais – Brazil (S21°45'38.8", W43°21'59.3" elevation 860 m) in February 2007 and dried at room temperature, arranged in thin layers, on a rack with wire-nettings that allow free aeration of plant material. The essential oils obtained from those plants were extracted by hydro-distillation using a Clevenger apparatus during 2 h. The extracted essential oils (Essential Oil of *L. camara* – EOLC and Essential Oil of *B. dracunculifolia* – EOBD) were kept at -20 °C in an amber flask.

Preparation of Mycobacterial Suspension

The species of *Mycobacterium tuberculosis* Complex (Table 2) were seeded on Lowenstein-

Jensen (LJ) culture medium, kept at 37°C, and 21 days afterwards colonies were harvested to form a suspension of turbidity equal to 1.0 McFarland Standard. From this suspension a second one was prepared diluting the first one 1:20 (v/v) in Middlebrook 7H9 broth (BD-lot.2112134-USA) enriched with Middlebrook OADC (Oleic Albumin Dextrose Catalase - SIGMA-ALDRICH). This second suspension was used for assays evaluating antimycobacterial activities.

The species of Non-tuberculous mycobacteria (Table 2) were seeded on LJ and after seven days the colonies were harvested to form a suspension of turbidity equal to 1.0 McFarland Standard. From this suspension a second one was prepared diluting the first one 1:25 (v/v) in Muller Hinton Broth (Becton Dickinson and Company).

Evaluation of antimicrobial activity

The tests of the potential antimycobacterial activity of the plant materials (extracts, essential oils and some fractions) and the standard antibiotics [rifampicin (Lot 780773 – SIGMA) and ciprofloxacin (Lot 17850 – ALDRICH Chemistry)] were performed in plates with 96 wells using redox reaction of sodium resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide - Resazurin Sodium Salt Powder Acros Organic N.V., Geel, Belgium) according to Machado et al. (2012a; 2012c).

Each sample of 5000 µg of prepared plant materials (Table 1) was diluted with 100 µL of DMSO (dimethyl sulfoxid - SIGMA) and 900 µL of supplemented 7H9 (liquid culture medium added with OADC) for the assays with mycobacteria of the *Mycobacterium tuberculosis* Complex (Table 2), or 900 µL of Mueller Hinton Broth (Becton Dickinson and Company) for the assays with Non-tuberculous mycobacteria (Table 2) (Machado et al., 2012c) The solution of rifampicin was prepared using ethylene glycol (Difco) as diluent. The rifampicin concentration of the solution was 256µg/ml and the final percentage of ethylene glycol in the solution was 0.1%. The solution of ciprofloxacin was prepared using 7H9 as diluent. The solutions of plant materials, the DMSO solution (10%), the ethylene glycol and the antibiotics solutions were tested simultaneously in triplicates in different wells of microplates and the DMSO solution

and ethylene glycol solution were not able to inhibit the mycobacterial growth. The final concentrations of plant material solutions evaluated in microplates were: 1250 µg/mL; 625 µg/mL; 312.5 µg/mL and so on until 4.88 µg/mL. The final concentrations of rifampicin solutions tested in microplates were: 4.00 µg/mL; 2.00 µg/mL; 1.00 µg/mL and so on until 0.03 µg/mL. The final concentrations of ciprofloxacin solution tested in microplates were 0.50 µg/mL; 0.25 µg/mL; 0.125 µg/mL and so on until 0.0039 µg/mL. The procedures of dilution of the antibiotic followed the description in Machado et al. (2012c).

The wells on the border of each microplate were filled with water in order to reduce the evaporation of the samples which occupied the inner part of the microplates.

To obtain the cited concentrations, all wells of the remaining inner part of the microplates were firstly filled with 100 µL of culture medium. Then all wells of the first line of the inner part (second line of the total plate) received 100 µL of the prepared solution of the plant material or antibiotic solutions. Next a serial dilution was created by passing 100 µL from top to bottom along the rows thus reducing the concentrations always by a factor 1:2 from one line to the following. Finally all wells of the inner part received 100 µL of the mycobacterial suspension. The filled microplate were kept at 37 °C. The readout was performed after 7 days, dropping 10 µL of resazurin (0.01% - diluted in ethylene glycol and sterile distilled water) into the wells. The change of color from blue to pink indicated the mycobacterial growth.

The minimal inhibitory concentration (MIC) less than 200 µg/mL were considered potential antimycobacterial agents (Tosun et al., 2004).

The MIC values were determined based on the means of triplicates. In addition, all procedures

were repeated at least three times with periodicity of 2 weeks, performing each one in triplicate. When a repetition of an experiment gave a deviating result, the number of repetitions was increased in order to guarantee the reliability of the results.

RESULTS AND DISCUSSION

The MIC values of ciprofloxacin against tested non-tuberculous mycobacteria (*Mycobacterium smegmatis*: 0.014 ± 0.005 µg/mL, *M. chelonae*: 0.015 ± 0.010 µg/mL, *M. abscessus*: 0.2 ± 0.2 µg/mL) and the MIC values of rifampicin against evaluated *Mycobacterium tuberculosis* Complex (*M. tuberculosis*: 0.051 ± 0.031 µg/mL and *M. bovis*: 0.030 ± 0.003 µg/mL) demonstrated the occurrence of susceptibility to all tested mycobacteria, as was expected. These findings are in accordance with results in the literature (Cavusoglu et al., 2012; Ribeiro et al., 2004).

The evaluation of antimycobacterial activity of plant materials showed promising results, since four of the investigated bacterial species were susceptible to some of the plant samples tested. The data are demonstrated in (Tables 3 and 4).

Pterodon emarginatus was the most promising species evaluated, showing significant toxic action by the HEPE against *M. smegmatis* (78.125 µg/mL) and *M. chelonae* (39.1 µg/mL). According to Dutra et al. (2012b) the previous phytochemical analysis of HEPE revealed occurrence of tannins, flavonoids and leucoanthocyanidins. These chemical classes of substances were absent in MEPE, which was not able to inhibit the mycobacterial growth (Table 3) suggesting that the actives belong to one or more of the cited phenolic chemical classes.

M. bovis is often used as a model to investigate *M. tuberculosis*. *M. smegmatis* has been

TABLE 3. Minimal Inhibitory Concentrations of mycobacterial growth determined from *in vitro* tests with fruits of *P. emarginatus*

Plant materials	M. smegmatis	M. chelonae	M. abscessus	M. tuberculosis	M. bovis
	(MIC-µg/mL)				
HEPE	78.125	39.1	> 1250	312.5	625
EAEPE	625	> 1250	> 1250	> 1250	625
BEPE	> 1250	> 1250	> 1250	> 1250	> 1250
MEPE	> 1250	> 1250	> 1250	> 1250	625
EOPE	> 1250	729	> 1250	46	78.125
		I 312.5 II 625 III 1250		I 19.55 II 39.1 III 78.125	

Notes: HEPE - Hexane Extract of *P. emarginatus*; EAEPE - Ethyl-Acetate Extract of *P. emarginatus*; BEPE - Butanol Extract of *P. emarginatus*; MEPE - Methanol Extract of *P. emarginatus*; EOPE - Essential Oil of *P. emarginatus*. MIC values were obtained as mean values of three assays. As the results of all triplicates were uniform, the table contains no standard deviation. In two cases the three assays I, II and III gave different values and they were listed separately. Promising results with MIC values smaller than 200µg/mL are shown in boldface.

TABLE 4. Minimal Inhibitory concentrations of mycobacterial growth determined from in vitro tests with *C. asiatica* leaves, *L. camara* leaves and *B. dracunculifolia* aerial parts

Plant materials	M. smegmatis	M. chelonae	M. abscessus	M. tuberculosis			M. bovis
	(MIC- $\mu\text{g/mL}$)						
AECA	> 1250	> 1250	> 1250	> 1250			> 1250
MECA	> 1250	> 1250	> 1250	> 1250			> 1250
HFCA	> 1250	78.125	> 1250	> 1250			> 1250
CHFCA	> 1250	> 1250	> 1250	> 1250			> 1250
EOLC	> 1250	> 1250	> 1250	> 1250			> 1250
EOBD	> 1250	> 1250	> 1250	729			> 1250
				I	II	III	
				312.5	625	1250	

Notes: AECA - Aqueous Extract of *C. asiatica*; MECA - Methanol Extract of *C. asiatica*; HFCA - Hexane Fraction of *C. asiatica*; CHFCA - Chloroform-Hexane Fraction of *C. asiatica*; EOLC - Essential Oil of *L. camara*; EOBD - Essential Oil of *B. dracunculifolia*. MIC values were obtained as mean values of three assays. As the results of all triplicates were uniform, the table contains no standard deviation. In one case the three assays I, II and III gave different values and they were listed separately. Promising result with MIC value smaller than 200 $\mu\text{g/mL}$ is shown in boldface.

used as model for *M. bovis* and *M. tuberculosis* (Altaf et al., 2010). However, the results obtained (78.125 $\mu\text{g/mL}$, 312.5 $\mu\text{g/mL}$ and 625 $\mu\text{g/mL}$, the MIC values of HEPE against *M. smegmatis*, *M. tuberculosis* and *M. bovis*, respectively) indicate that such use is of limited value, since the active substances in the extract HEPE that inhibit the growth of *M. smegmatis* do not affect *M. bovis* and *M. tuberculosis*.

Despite the low antimycobacterial activity of HEPE against *M. tuberculosis* and *M. bovis* this extract deserves attention, since previous study of the biological properties of HEPE revealed it to be toxic against *Artemia salina* (LD50 = 8.06 $\mu\text{g/mL}$), but not against mice, when it was used topically as ointment (Dutra, 2008).

The inhibitory action of the EOPE on the *M. tuberculosis* development *in vitro* was significant according to Tosun et al., (2004), presenting a mean MIC value of 46 $\mu\text{g/mL}$. As can be seen in Table 3, this mean value is composed of MIC values that increase with time, reaching a saturation value of 78.125 $\mu\text{g/mL}$. This suggests that EOPE contains at least two active substances, one of them is unstable on a scale of time of a few weeks and the other one seems to be stable. The stable compound seems to affect *M. bovis* in the same way, but the inhibitory action of the unstable ones are limited to *M. tuberculosis*.

The results of Table 3 were experimentally verified at least three times (each one in triplicates) with periodicity of two weeks. In the cases where a variable behavior was found, such as EOPE against *M. chelonae* and EOPE against *M. tuberculosis*, 6 and 7 assays were performed respectively. This precaution was used to test the reliability, while revealing unstable substances that may be involved in some activities.

Previous investigation of the currently used EOPE revealed its chemical composition by GC/MS, showing the following major compounds: *trans*-caryophyllene (35.9%), β -elemene (15.3%) and germacrene D (9.8%) (Dutra et al., 2012a).

According to Kiliç (2006), the *trans*-caryophyllene was able to inhibit the growth of *M. tuberculosis*, but the MIC value was 514 $\mu\text{g/mL}$, a MIC value higher than the value founded for EOPE (46 $\mu\text{g/mL}$) in the present work. This finding suggests that the potential activity of the EOPE may not be exclusively due to the presence of *trans*-caryophyllene, but due to a minor constituent present in EOPE.

According to the PubMed and Scielo databases, the potential of essential oil from leaves of *L. camara* (EOLC) against mycobacteria was evaluated for the first time. The tests revealed that this essential oil was not able to inhibit the growth of evaluated *Mycobacterium* species until 1250 $\mu\text{g/mL}$ of EOLC (Table 4).

The composition of the currently used EOLC was described in a previous study that showed its leishmanicidal potential (Machado et al., 2012b). This previous study presented germacrene D, caryophyllene and farnesene as the major compounds. Thus, despite the absence of antimycobacterial activity of EOLC, the described composition of EOLC and EOPE (Dutra et al., 2012a; Machado et al., 2012b) suggests that the germacrene D is not related to the antimycobacterial activity revealed by EOPE in this work, since both essential oils presented germacrene D as one of the major compounds.

The essential oil of *B. dracunculifolia* (EOBD) was investigated against *Mycobacterium* species. According to the results obtained this

essential oil does not represent a promising source for antimycobacterial drugs, since its MIC value was 729 µg/mL against *M. tuberculosis*, exceeding the limit of MIC value determined as 200 µg/mL by Tosun et al., (2004).

On the other hand, the low toxicity of this plant material has been described in other works revealing no toxic alteration in mice that received approximately 11mg/mL of different extracts of *B. dracunculifolia* from different parts of the plant (Fabri et al., 2011; Lemos et al., 2007; Menezes, 2005). Furthermore, the authors did not find any register in the literature of intoxication with this plant by the population.

The *M. chelonae* was susceptible to HEPE and HFCA presenting the following MIC values, 39.1 µg/mL and 78.125 µg/mL, respectively. In view of the activity of HEPE against *M. chelonae* and *M. smegmatis* and of the toxic action of HFCA only against *M. chelonae*, it is possible to conclude that there are at least two active substances in HEPE able to inhibit the growth of these mycobacteria. These active substances present in the HEPE, according to Dutra et al. (2012b), belong to the following chemical classes of secondary metabolism: tannins, flavonoids and leucoantocyanidins.

Considering the phytochemical analysis of MECA performed by Silva (2008) and the fact that HFCA is derived from MECA, it can be concluded that their active compounds are included in one of the following chemical classes: flavonoids, triterpenic glycosides, coumarins and tannins. Additionally, despite the verified antimycobacterial activity of HFCA, the MECA was not able to inhibit the growth of evaluated mycobacteria. This fact can be explained considering the preparation performed to obtain MECA. During the hot extraction with methanol, both polar and apolar components were extracted. Thus MECA is composed of a mix of components of different polarities. When the preparation of a fraction from MECA was performed using the hexane, apolar compounds were concentrated. Thus, the absence of antimycobacterial activity of MECA and the presence of antimycobacterial activity of HFCA can be related to a greater amount of the active substance in the tested volume of the latter plant material than in the evaluated volume of MECA.

According to Andrade et al. (2008), the high presence of lipids in the cell wall of mycobacteria leads apolar substances to present a higher probability of acting against these microorganisms. Thus, the presence of antimycobacterial activity in EOPE, HEPE and HFCA, which are composed of apolar substances, are in accordance with the literature.

The *M. chelonae* is classified as a component of the *M. fortuitum* complex, that is closely related

to *M. abscessus* and *M. immunogenum*, which is sometimes known as the *M. chelonae/abscessus* group (Brown-Elliott & Wallace, 2005).

Despite the proximity of *M. abscessus* to *M. chelonae*, the results show differences between them, since *M. abscessus* was not susceptible to the active substances against *M. chelonae* present in HEPE and HFCA.

The results showed that none of the tested plant materials were able to inhibit the growth of *M. abscessus*. These data support the continuous need for research about new antimicrobial drugs against *M. abscessus*, which is often known as the most pathogenic and resistant non-tuberculosis *Mycobacterium* associated with pulmonary and soft tissue injuries (Griffith et al., 2007; Thomson et al., 2013).

CONCLUSIONS

The present work showed new potential sources of antimycobacterial drugs and pointed to *Pterodon emarginatus* as the most promising plant species. Comparing the results obtained for EOLC and EOPE to the literature records, we suggest that some compounds found in the essential oil from *P. emarginatus* fruits, such as the *trans*-caryophyllene and the germacrene D, are not responsible for the inhibitory action of the mycobacterial growth. Additionally, the present work presented one more biological property for *Centella asiatica*, since the hexane fraction of methanol extract from its leaves was active against *Mycobacterium chelonae*, a relevant non-tuberculosis *Mycobacterium*.

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