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Original Paper

Anti-mycobacterial and anti-inflammatory activity of *restinga* plants: a dual approach in searching for new drugs to treat severe tuberculosis

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Abstract

Tuberculosis (TB) still constitutes a threat to public health in various regions of the world. The existing treatment is long and has many side effects. The need to identify new anti-TB compounds and also adjuvants to control exacerbated inflammation in severe TB cases is relevant. Therefore, the objective of this study was to evaluate the anti-mycobacterial activity of extracts and fractions *in vitro* from plant species collected in the *Restinga* of Jurubatiba, in Rio de Janeiro state, Brazil. In addition, to verify their immunomodulatory action and cytotoxicity on macrophages. The dichloromethane fraction of *Kielmeyera membranacea* and *Eremanthus crotonoides* showed the lowest MIC_{50} against *Mycobacterium bovis* BCG (0.95 ± 1.08 and 2.17 ± 1.11 µg/mL, respectively) and *M. tuberculosis* $H_{37}Rv$ (4.38 ± 1.19 and 15.28 ± 1.21 µg/mL, respectively). They were also able to inhibit the NO and TNF-α production in LPS-stimulated macrophages, without being toxic to cells. Using gas chromatography analysis coupled with mass spectrometer it was possible to suggest the presence of fatty acids and terpenes in the most promising fractions. Those compounds have been described for their anti-mycobacterial activity. These results have enabled identifying *Kielmeyera membranacea* and *Eremanthus crotonoides* as the most promising studied species in searching for new anti-TB compounds with dual activity.
Key words: anti-mycobacterial, immunomodulatory, *restinga*, terpenes, tuberculosis.

Resumo

A tuberculose (TB) ainda representa um problema de Saúde Pública em várias regiões do mundo. O tratamento existente é longo e apresenta diversos efeitos adversos. Neste contexto, é relevante a necessidade de identificar novas substâncias anti-TB e complementares ao controle do processo inflamatório exacerbado em quadros severos da doença pulmonar. O objetivo deste trabalho foi avaliar, *in vitro*, extratos e frações de espécies vegetais coletadas na Restinga de Jurubatiba, quanto a sua atividade antimicobacteriana, assim como verificar a ação imunomoduladora e citotóxica em macrófagos. As frações em diclorometano de *Kielmeyera membranacea* e *Eremanthus crotonoides* apresentaram os menores CIM_{50} contra *Mycobacterium bovis* BCG (0,95 ± 1,08 e 2,17 ± 1,11 µg/mL; respectivamente) e *M. tuberculosis* $H_{37}Rv$ (4,38 ± 1,19 e 15,28 ± 1,21 µg/mL; respectivamente). Essas também foram capazes de inibir a produção de NO e TNF-α em macrófagos estimulados por LPS, sem serem tóxicas para as células. Através de análise por cromatografia em fase gasosa acoplada ao espectrômetro de massas foi possível sugerir a presença de ácidos graxos e terpenos nas frações mais promissoras, substâncias estas descritas por apresentarem atividade antimicobacteriana. Estes resultados permitiram a identificação de *Kielmeyera membranacea* e *Eremanthus crotonoides* como as espécies mais promissoras desse estudo, tendo em vista a busca de novos fármacos anti-TB com dupla atividade.
Palavras-chave: anti-micobacteriano, imunomodulatório, restinga, terpenos, tuberculose.

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Introduction

Tuberculosis (TB) is an infectious disease responsible for 1.2 million deaths in 2018 and an additional 0.25 million deaths resulting from TB among human immunodeficiency virus (HIV)-positive people (WHO 2019). The World Health Organization estimated 10 million new cases, with 484,000 being rifampicin or multidrug-resistant TB (MDR-TB) (WHO 2019), where approximately one in five tuberculosis isolates worldwide are resistant to at least one major first-line drug (Dheda *et al*. 2017).

Furthermore, in the cases of severe forms of TB such as military TB or tuberculous meningitis, additional anti-inflammatory therapy is required to prevent excessive inflammation (Pai *et al*. 2016; Zhang *et al*. 2017). Macrophages are the main host cells for mycobacteria which play an important role and are a potential target for modulating improper or excessive production of inflammatory mediators, which contributes to the pathogenesis of tuberculosis (Koul *et al*. 2004; Garlanda *et al*. 2007).

Thus, there is an important need to explore new treatment strategies and natural products are an important source of antibacterial compounds (Harvey *et al.* 2010). Different plant species and isolated compounds have shown significant *in vitro* anti-mycobacterial activity (Okunade *et al.* 2004; Coop & Pearce 2007; Salomon & Schmidt 2012). Potential dual anti-mycobacterial and anti-inflammatory activity has also been studied as an alternative and shown promising *in vitro* and *in vivo* results (Machado *et al*. 2014; Ventura *et al*. 2015a,b,c).

The abundance of Brazilian flora provides great possibilities for finding novel anti-TB compounds. The *restinga* vegetation has great biodiversity among Brazilian ecosystems and covers around 79% of the coastal sandy plains. This biodiversity is likely due to a combination of physical and chemical factors such as high temperature, soil salinity, extensive deposition of salt and high exposure to light (Cogliatti-Carvalho *et al.* 2001). These conditions favor a great diversity of habitats which are colonized by a wide variety of plant, animal and microorganism communities. In turn, this leads to differentiated production of secondary metabolites with the aim of environmental adaptation (Amaral *et al*. 2013). In the present study, the search for active compounds was based on random selection from *Restinga* of Jurubatiba National Park flora, located in the Northeast of the state of Rio de Janeiro (22°23'S, 41°45'W), Brazil. The distribution of these species in the studied area was taken into account in this process, and only abundant species were selected.

The first objective of the present work was to determine the activity of *Tapirira guianensis* Aubl., *Mandevilla moricandiana* (A.DC.) Woodson, *Eremanthus crotonoides* (DC.) Sch.Bip. (= *Vernonia crotonoides*), *Kielmeyera membranacea* Casar., *Stachytarpheta schottiana* Schauer, *Vitex polygama* Cham. and *Tocoyena bullata* (Vell.) Mart. from the *restinga* of Jurubatiba against avirulent *Mycobacterium bovis* bacille Calmette-Guérin (BCG) in order to evaluate the usefulness of the non-virulent vaccine strain as a model for detecting activity against the virulent *Mycobacterium tuberculosis* H_{37}Rv strain. This non-virulent *M. bovis* is an attenuated strain, but closely related to *M. tuberculosis* (Mahairas *et al*. 1996). Thus, *M. bovis* BCG could be used to provide useful information for screening studies.

Secondly, the modulation of pro-inflammatory mediators such as nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α) and the cytotoxicity effect in the macrophages were evaluated. These mediators can indicate an anti-inflammatory profile for the samples, and together with anti-mycobacterial activity data can enable discovering compounds with dual activity.

In a third component, infected macrophages were treated with samples to confirm the role of these mediators and anti-mycobacterial activity together, and the production of NO, TNF-α was measured, as well as the cytotoxic effect. In screening studies, this could reinforce the rationale on the role of mediators during mycobacterium infection, helping to identify natural products with both anti-mycobacterial and anti-inflammatory activity. While some work has been done in this regard, further data are required to support this reasoning.

Materials and Methods

Reagents

Cell culture reagents were purchased from Gibco/Invitrogen (Grand Island, NY, USA). Mycobacteria-specific Middlebrook 7H9 and 7H10 media were obtained from Difco (Detroit, MI, USA); albumin dextrose catalase (ADC) and oleic albumin dextrose catalase (OADC) supplements were from BD Biosciences (BD, Sparks, MD, USA).

Murine recombinant TNF-α from Biosource®. Lipopolysaccharide (LPS) from serotype 0111:B4 *Escherichia coli*, N^G-monomethyl-L-arginine acetate salt (L-NMMA - 98% purity) (cod. M7033), rifampicin (cod. R7382) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The samples and rifampicin were dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich); other reagents indicated for cell treatment were dissolved in sterile phosphate buffered saline (PBS) and sterilized by passage through 0.22 μm nylon filters (Corning Inc., Wilkes-Barre, PA, USA).

Plant materials and preparation of extracts and fractions

Leaves of the 7 plant species were collected: BRASIL. RIO DE JANEIRO: Quissamã, Restinga de Jurubatiba National Park, 22°11'53.8"S, 41°27'48.2"W, 10 m above sea level, 20.II.2011, fl., *T.U.P. Konno* (RFA), (Herbarium of the Federal University of Rio de Janeiro). The voucher specimen codes and relevant information for each species are described in Table 1. This study complied with all relevant federal guidelines and institutional policies related to the botanical material for research purposes, Sisbio/ICMBio number: 62422-11 and SisGen number: AAA989F.

The leaves were air-dried at room temperature, triturated and extracted by maceration for 24 h at room temperature with the described solvents (Tab. 1). The solvents were renewed five times, completing the extraction after 120 h. After extraction, the materials were concentrated under vacuum in a rotary evaporator at temperatures below 45 °C and the solvent was eliminated to leave the crude extract. The crude extract was lyophilized, dissolved in water:methanol (H_2O:MeOH) (1:9) and fractions were obtained by liquid-liquid partition, using solvents with increasing polarity: firstly, hexane (Hex - 1/3 of the total volume by 3 times), then MeOH phase was concentrated, dissolved in H_2O and partitioned with dichloromethane (DCM - 1/3 of the total volume by 3 times), aqueous phase was partitioned with ethyl acetate (EtOAc - 1/3 of the total volume by 3 times), at long last aqueous phase was partitioned butanol (BuOH - 1/3 of the total volume by 3 times) and the last residual aqueous phase (Aq) was concentrated and lyophilized. This procedure was repeated for each plant species and the respective fraction codes are shown (Tab. 1).

Analysis by gas chromatography (GC) coupled to mass spectrometry (MS)

Fractions were submitted to Shimadzu GC-MS/QP2010 equipment using capillary columns of fused silica RTx-5MS (30 m × 0.25 μm) from Restek Corporation Pennsylvania USA). Injector temperature: 270 °C (SPLIT). Mass detector conditions: ionization source, 200 °C; Interface, 230 °C. The column temperature was maintained at 60 °C for 1 min and then subsequently increased to 280 °C at a rate of 15 °C/min and held for 10 min. Helium was used as carrier gas with a flow rate of 1.1 mL/min. The fractions (1 mg) were derivatized using 100 μL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) for 30 min. After 15 min of standing the vial with the fraction, MSTFA was thoroughly vortexed for 2 minutes and then returned to stand. The derivatized fractions were dissolved in dichloromethane at 1 mg/mL, and 1 μL was injected with an automatic injector (Carneiro *et al*. 2018).

Anti-mycobacterial activity

Two mycobacterial strains were used in this study: avirulent *Mycobacterium bovis* BCG, Moreau vaccine strain, and virulent *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294). Mycobacterial strains were grown in suspension in Middlebrook 7H9 medium, supplemented with 0.5% glycerol, 0.05% Tween-80 and 10% of ADC at 37 °C under biosecurity level 3 containment conditions. The suspension densities were measured by spectrophotometry at 600 nm and corresponding concentrations were determined for each serial dilution strain with plating on Middlebrook 7H10 agar plates supplemented with 0.5% glycerol and 10% of OADC. During the middle logarithmic growth phase, the bacterial suspensions were plated on a 96-well microplate (1×10^6 colony forming unit - CFU/well) in the presence of plant samples at concentrations of 100, 20, 4 and 0.8 μg/mL or rifampicin (ranging from 0.001 to 0.03 μg/mL for *M. bovis* BCG and from 0.008 to 1 μg/mL for *M. tubelculosis* H_{37}Rv). The plates were sealed and incubated at 37 °C and 5% of carbon dioxide (CO_2) for 7 days for *M. bovis* BCG or 5 days for *M. tuberculosis* strains. The mycobacterial cultures were incubated after these periods for 3 h with MTT solution (5 mg/mL) and then treated overnight with lysis buffer (20% w/v Sodium dodecyl sulfate (SDS)/50% dimethylformamide (DMF) in distilled water - pH 4.7) (Gomez-Flores *et al*. 1995). The

Table 1 – Relevant information of the studied species and evaluation of inhibition of *Mycobacterium bovis* BCG growth when treated with extracts and fractions from *restinga* species.

Family and botanical name	Common name[#]	Voucher number	Part used and solvent extractor	Samples	Sample code	*Mycobacterium bovis* BCG	
						MIC_{50} ± SD (µg/mL)	Maximum inhibition (%)
Anacardiaceae	Micumi	RFA38757	Leaves	Crude Extract	Tg CE	>100	22.33±0.0
				Fractions			
Tapirira guianensis Aubl.			Methanol	Hexane	Tg HEX	86.53±1.29	56.39±0.90
				Dichloromethane	Tg DCM	22.47±1.12	77.83±1.32
				Ethyl acetate	Tg EtOAc	49.16±1.38	79.06±0.00
				Butanol	Tg BuOH	80.62±1.30	54.17±1.62
				Aqueous	Tg Aq	>100	43.21±1.97
Apocynaceae	Chifre-de-veado	RFA38748	Leaves	Crude Extract	Mm CE	>100	18.74±0.00
				Fractions			
Mandevilla moricandiana (A. DC.) Woodson			Ethanol/water (7:3, v/v)	Hexane	Mm HEX	>100	26.65±4.89
				Dichloromethane*	Mm DCM	11.97±1.23	93.81±0.12
				Ethyl acetate	Mm EtOAc	18.44±1.16	83.25±1.02
				Butanol	Mm BuOH	>100	16.67±3.37
				Aqueous	Mm Aq	>100	43.86±4.02
Asteraceae	Papel sanitário das índias	RFA38749	Leaves	Crude Extract*	Vc CE	4.42±1.38	94.64±1.26
				Fractions			
Eremanthus crotonoides (DC.) Sch.Bip.			Ethanol/water (8:2, v/v)	Hexane	Ec HEX	20.14±1.21	80.61±2.88
				Dichloromethane*	Ec DCM	2.17±1.11	93.11±0.07
				Ethyl acetate	Ec EtOAc	21.67±1.49	70.98±3.72
				Butanol	Ec BuOH	84.42±1.69	50.70±0.0
				Aqueous	Ec Aq	90.26±2.01	56.62±2.82
Calophyllaceae	Pequiá-branco	RFA38752	Leaves	Crude Extract	Km CE	29.92±1.14	74.60±2.34
				Fractions			
Kielmeyera membranacea Casar.			Ethanol/water (7:3, v/v)	Hexane	Km HEX	>100	46.31±2.42
				Dichloromethane*	Km DCM	0.95±1.08	96.83±0.14
				Ethyl acetate	Km EtOAc	>100	10.27±5.29
				Butanol	Km BuOH	30.58±1.20	77.14±1.38
				Aqueous	Km Aq	41.10±1.25	59.88±2.04

Family and botanical name	Common name[#]	Voucher number	Part used and solvent extractor	Samples	Sample code	*Mycobacterium bovis* BCG	
						MIC_{50} ± SD (µg/mL)	Maximum inhibition (%)
Lamiaceae	Carabuçu	RFA38750	Leaves	Crude Extract	Vp CE	22.64±1.28	81.55±1.54
				Fractions			
Vitex polygama Cham.			Ethanol/water (8:2, v/v)	Hexane	Vp HEX	13.43±1.43	89.09±0.84
				Dichloromethane*	Vp DCM	8.90±1.26	91.65±0.54
				Ethyl acetate	Vp EtOAc	>100	50.55±7.68
				Butanol	Vp BuOH	3.40±1.90	83.25±3.30
				Aqueous	Vp Aq	>100	38.85±2.28
Verbenaceae	Gervão-mirim	RFA38759	Leaves	Crude Extract	Ss CE	78.77±1.32	54.09±1.26
				Fractions			
Stachytarpheta schottiana Schau			Ethanol/water (8:2, v/v)	Hexane*	Ss HEX	1.52±1.30	97.42±0.00
				Dichloromethane	Ss DCM	44.52±1.13	75.26±5.36
				Ethyl acetate	Ss EtOAc	29.36±1.23	76.68±0.72
				Butanol	Ss BuOH	32.41±1.30	71.03±4.02
				Aqueous	Ss Aq	>100	37.32±4.20
Rubiaceae	Jenipapo-da-praia	RFA38974	Leaves and Flowers	Crude Extract	Tb CE	>100	27.01±2.20
				Fractions			
Tocoyena bullata (Vell.) Mart.				Hexane	Tb HEX	>100	36.08±3.89
			Ethanol	Dichloromethane	Tb DCM	42.06±1.13	73.99±1.04
				Ethyl acetate	Tb EtOAc	58.24±1.50	79.76±4.01
				Butanol	Tb BuOH	>100	3.75±2.46
				Aqueous	Tb Aq	66.38±1.32	57.25±2.64

Antibiotic rifampicin was used at concentrations of 0.001, 0.003, 0.01 and 0.03 µg/mL, demonstrating 16.78±4.88%, 71.58±2.13%, 98.85±1.06% and 99.63±0.32% growth inhibition, respectively; # = Santos *et al.* 2010; ∗ = The most promising activities; Maximum inhibition (%) = the highest percentage of inhibition obtained at the concentrations tested; MIC_{50} = lowest concentration at which 50% of the growth was inhibited.

resulting optical densities of the samples were measured in microplate reader at 570 nm. A mycobacterial suspension treated with rifampicin (Sigma-Aldrich ≥ 99% purity) was used as a positive control, while untreated mycobacterial suspensions were used as a negative control.

Culture and treatments of LPS-activated RAW 264.7 cells

Murine macrophages RAW 264.7 (ATCC, TIB-71) were cultured in Dulbecco Modified Eagle medium (DMEM-F12) supplemented with 10% Fetal bovine serum (FBS) and Gentamicin (0.2%) in 5% CO_2 atmosphere at 37 °C. Cells were seeded in 96-well microplates (5×10^4 cells/well) for the experiments and incubated for 24 h. Cells were stimulated after this period with 1 µg/mL LPS (*Escherichia coli* 0111:B4; Sigma-Aldrich) and treated with plant samples at concentrations of 100, 20, 4 and 0.8 µg/mL for 24 h. Next, culture supernatants were collected for NO and TNF-α assays (Araujo *et al*. 2017).

Determination of nitric oxide (NO) production

In the nitric oxide experiments, an inhibitor of the inducible isoform of nitric oxide synthase (iNOS), L-*N*MMA was used as a positive control at 20 µg/mL. Stimulated and untreated macrophages were used as a negative control. Nitrite, which is a stable NO metabolite, was assessed by the Griess method (Griess 1879). The nitrite concentration was calculated from a $NaNO_2$ standard curve. The optical density was measured in microplate reader at 540 nm.

Determination of tumor necrosis factor-alpha (TNF-α) production

Tumor necrosis factor-alpha (TNF-α) was measured by an L929 fibroblast (ATCC, CCL-1) bioassay based on the sensitivity of L929 cells to the cytotoxic effect of TNF-α. To do so, the L929 cells were seeded in 96-well microplates (2.5×10^4 cells/well). The resulting cell monolayers after 24 h of incubation were treated with the macrophage culture supernatants in the presence of actinomycin D (2 µg/mL). After an additional 24 h incubation, the L929 cell viability was assayed by the MTT assay (Mosmann 1983). The cytokine concentrations were determined by using a recombinant mouse cytokine to obtain a standard curve correlating cellular viability and TNF-α concentration. The optical density of each well was measured at 570 nm employing a microplate reader.

Macrophage cytotoxicity assay

The cytotoxic effects of plant samples on RAW 264.7 cell viability in cultures stimulated with LPS were determined using the lactate dehydrogenase (LDH) release assay as previously described (Muzitano *et al*. 2006). First, the cells (5×10^4 cells/well) were seeded in 96-well microplates and then treated with plant samples at 100, 20, 4 and 0.8 µg/mL for 24 h. Cytoplasmic LDH enzyme release into cell culture supernatants was detected using a commercial LDH kit (Doles, GO, Brazil). Cell lysates obtained through the treatment with 1% Triton X-100 were used as a positive control. DMSO was used as solvent for the sample dilutions, and was tested in parallel as control. Stimulated and untreated cells supernatants were used as a negative control.

Infection of macrophage and quantification of intracellular growth

RAW 264.7 macrophages were plated (1×10^5 cells/well) in antibiotic-free DMEM-F12 medium supplemented with 10% FBS and incubated for 24 h. Mycobacterial suspensions were sonicated prior to infection for 1 minute to disperse clumps and optical densities were adjusted to 0.1. The macrophage cultures were infected at a multiplicity of infection (MOI) of 1:1 (macrophage:mycobacterium). Phagocytosis was allowed to progress for 3 h. Extracellular mycobacteria were removed after 3 h by washing with PBS 1X and the infected cell monolayers were treated for 4 days with plant samples or rifampicin. Macrophage viability was monitored by LDH assay and was over 80% in all experiments. Cells were lysed on day 4 after infection with 1% saponin to release intracellular bacteria. Lysate aliquots were diluted 10-fold in PBS, plated in triplicate on 7H10 agar plates and incubated at 37 °C. Total CFU were determined after 21 days (Lasunskaia *et al*. 2010).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and the Tukey test for multiple range tests, employing GraphPad Prism 5 software to assess statistical significance between data groups. The results were considered statistically significant for $p < 0.05$. The half maximal inhibitory concentration (IC_{50}), lowest concentration at which 50% of the growth was inhibited (MIC_{50}) and 50% cytotoxic concentration

(CC_{50}) values were calculated by non-linear regression analysis of log[concentration]/inhibition curves by GraphPad Prism 5 Software, applying a sigmoidal dose-response variable slope curve fitting using the different percentages obtained for each corresponding concentration in triplicate and were expressed as means with a corresponding 95% confidence interval.

Results and Discussion

Crude extracts and fractions of *Tapirira guianensis*, *Mandevilla moricandiana*, *Eremanthus crotonoides* (=*Vernonia crotonoides*), *Kielmeyera membranacea*, *Stachytarpheta schottiana*, *Vitex polygama* and *Tocoyena bullata* were screened against *Mycobacterium bovis* BCG and showed mean MIC_{50} values ranging from 0.95 to greater than 100 μg/mL (Tab. 1). Higher inhibitory activities were observed for crude extract from *E. crotonoides* (94.64 ± 1.26%), hexane fraction from *S. schottiana* (97.42 ± 0.01%), dichloromethane fraction from *V. polygama*, *E. crotonoides*, *M. moricandiana* and *K. membranacea* (91.65 ± 0.54, 93.11 ± 0.07, 93.81 ± 0.12 and 96.83 ± 0.14%, respectively).

The plant species investigated in this study or species of the same genus have traditionally been used and/or reported with antibacterial activity or used for cough and fever and related symptoms for tuberculosis, among other diseases. The compounds isolated from *Tapirira guianensis* bark have shown activity against *Staphylococcus aureus* and *S. epidermidis* (Roumy *et al.* 2009). A study on the antibacterial activity of northern-peruvian medicinal plants reported *Mandevilla trianae* Woodson as an effective inhibitor of *Escherichia coli* (Bussmann *et al*. 2008). In addition, vine extract from *Mandevilla veraguasensis* Seem.) Hemsl. was able to boost the antimicrobial activity of human blood against *S. aureus* (Yaseen *et al*. 2017).

The extracts and isolated compounds from *Vernonia amygdalina* Delile flowers showed considerable antibacterial activities against different pathogenic bacteria (Habtamu & Melaku 2018). *Vernonia condensata* Baker, traditionally used to treat infectious processes and inflammation, also showed promising activity against *S. aureus*, providing scientific support to ethnopharmacological use (Silva *et al*. 2018).

Extracts from *Kielmeyera variabilis* Mart. & Zucc., *K. lathrophyton* Saddi, *K. neglecta* Saddi showed antibacterial activity, with the latter species being active against multidrug-resistant bacteria (Pinheiro *et al*. 2003; Toledo *et al*. 2011; Sousa *et al*. 2012). *Stachytarpheta indica* (L.) Vahl and *S. urticifolia* (Salisb.) Sims have also had their traditional uses as antimicrobials supported scientifically (Princely *et al*. 2013; Sreelatha *et al*. 2014).

Vitex negundo L., traditionally used to treat colds, coughing, and bacterial dysentery was identified as having anti-mycobacterial activity (Gupta *et al*. 2011). In another work, *Vitex* species, namely, *V. altissima* L.f., *V. diversifolia* Kurz *ex* C.B.Clarke, *V. peduncularis* Wall. *ex* Schauer and *V. trifolia* L., were investigated against bacterial strains, in which *V. peduncularis* was the most interesting species (Kannathasan *et al*. 2011). No previous anti-mycobacterial studies have been reported from *Tocoyena bullata*. However, phytochemical study of this species has been carried out by our group and phenolic compounds showed inhibitory activity in mast cells degranulation (Santos *et al*. 2019).

The plant species were found to be active against *M. bovis* BCG in the primary screening, and eleven samples showed more than 80% inhibition and were screened against a virulent strain of *Mycobacterium tuberculosis* $H_{37}Rv$. This strain was found to be more sensitive towards three plant species, and the results are compiled in Table 2. The most antimycobacterial activity was observed in the dichloromethane fraction from *K. membranacea* with 97.40 ± 1.99% of growth inhibition at 100 μg/mL and a MIC_{50} value of 4.38 ± 1.19 μg/mL (Tab. 2).

Six samples in our study showed inhibition ≥ 90% at 100 μg/mL against *M. bovis* BCG and three samples against *M. tuberculosis* $H_{37}Rv$. Our findings are in agreement with Tosun *et al*. (2004), where extracts which do not inhibit *M. tuberculosis* in at least 200 μg/mL are considered inactive. Active samples should have MIC values ≤ 128 μg/mL (Gu *et al*. 2004).

The production of pro-inflammatory mediators such as interleukin-1 (IL-1), IL-12, TNF-α and NO by the infected macrophages is generally essential for protection against mycobacteria (Garlanda *et al*. 2007). However, additional anti-inflammatory therapy is required to prevent excessive inflammation in the cases of severe forms of TB such as military TB or tuberculous meningitis and pericarditis (Zhang *et al*. 2017). In addition, anti-inflammatory therapy reduces mortality in patients exhibiting hyperinflammatory phenotype which could be determined by host genetic polymorphisms, increased bacterial virulence or specific comorbid states (Critchley *et al*. 2013).

Table 2 – Evaluation of inhibition of *Mycobacterium tuberculosis* $H_{37}Rv$ growth when treated with extracts and fractions from *restinga* species.

Botanical name	Samples	Sample code	MIC_{50} ± SD (µg/mL)	Maximum inhibition (%)
Mandevilla moricandiana	Dichloromethane	Mm DCM	>100	19.04±2.59
	Ethyl acetate	Mm EtOAc	>100	11.41±4.34
Eremanthus crotonoides	Crude Extract	Vc CE	48.11±1.29	94.38±0.96
	Hexane	Vc HEX	>100	23.66±1.64
	Dichloromethane	Vc DCM	15.28±1.21	94.89±0.60
Kielmeyera membranacea	Dichloromethane	Km DCM	4.38±1.19	97.40±1.99
Stachytarpheta schottiana	Hexane	Ss HEX	>100	34.03±1.26
Vitex polygama	Crude Extract	Vp CE	>100	34.76±1.59
	Hexane	Vp HEX	>100	46.07±3.97
	Dichloromethane	Vp DCM	78.32±1.47	50.47±0.30
	Butanol	Vp BuOH	>100	44.32±5.00

Antibiotic rifampicin was used at concentrations of 0.008, 0.04, 0.2 and 1 µg/mL, demonstrating 83.92±1.80%, 95.40±0.68%, 98.16±1.49% and 100.13±0.19% growth inhibition, respectively; MIC_{50} = lowest concentration at which 50% of the growth was inhibited; SD = standard deviation; Maximum inhibition (%) = the highest percentage of inhibition obtained at the concentrations tested.

According to these aspects, the eleven samples previously assayed against *M. tuberculosis* $H_{37}Rv$ were evaluated for inhibition of NO and TNF-α production in LPS-stimulated RAW 264.7 macrophage culture. All the samples were able to inhibit NO production by more than 50%, and six samples were able to inhibit TNF-α production by more than 50%. The *K. membranacea* and *E. crotonoides* species showed potential inhibitory capacity, with all IC_{50} values showing < 0.8 µg/mL for NO and 4.70 ± 1.84, 5.83 ± 1.40, 1.82 ± 1.77 µg/mL for TNF-α from Km DCM, Ec CE and Vc DCM, respectively (Tab. 3). This modulation could present benefits in treating severe tuberculosis.

A previous study with methanol stem extract of *Kielmeyera rugosa* Choisy demonstrated anti-hyperalgesic and anti-inflammatory effects, and these effects were associated with the inhibition of cytokine cascade generated by carrageenan and/or in decreasing the production of inflammatory mediators by significantly reducing the TNF-α and IL-1β (Melo *et al.* 2014). This suggests that other species of the *Kielmeyera* genus display anti-inflammatory activity and also inhibit TNF-α. Furthermore, *Vernonia cinerea* (L.) Less. species also exhibited strong inhibitory effects on nitric oxide production in LPS-stimulated RAW264.7 cells (Kuo *et al*. 2018)

The cytotoxic effect was evaluated in macrophages and the samples showed no significant cytotoxicity. The selectivity index (SI) was calculated for the three most active species of *K. membranacea*, *E. crotonoides* and *V. polygama*. The SI values calculated for DCM fractions from these species were > 100, 45 and 10 for *M. bovis* BCG, and > 20, 5 and 1 for *M. tuberculosis* $H_{37}Rv$, respectively. The selectivity index > 10 suggests that it will be safer and the sample can then be evaluated further (Orme 2001).

Mycobacterium bovis BCG in our study was sensitive for 28 samples, and four samples among these were active against *M. tuberculosis* $H_{37}Rv$, highlighting three active species of *K. membranacea*, *E. crotonoides* and *V. polygama*. These findings are highly significant since there is an impermeability associated with the complex cell wall structure.

Intracellular growth of *M. bovis* BCG in infected macrophages was also evaluated. The active dichloromethane fractions from *K. membranacea* and *E. crotonoides* at 100 µg/mL decreased mycobacterial growth from 7.7×10^4 to 2.6×10^4 CFU/mL (66.23 ± 3.67%) and from 11×10^4 to 8.5×10^4 CFU/mL (22.73 ± 1.29%), respectively. The rifampicin (positive control)

Table 3 – Inhibitory effects of extracts and fractions from studied species on production of NO and TNF-α by LPS-stimulated RAW 264.7 macrophages, and evaluation of cytotoxicity by LDH test.

Botanical name	Samples	Sample code	NO IC_{50} ± SD (µg/mL)	TNF-α IC_{50} ± SD (µg/mL)	CC_{50} ± SD (µg/mL)
Mandevilla moricandiana	Dichloromethane	Mm DCM	33.80±1.89	>100	90.38±1.32
	Ethyl acetate	Mm EtOAc	62.13±1.84	54.66±1.18	>100
Eremanthus crotonoides	Crude Extract	Vc CE	<0.8	5.83±1.40	>100
	Hexane	Vc HEX	<0.8	55.05±1.17	>100
	Dichloromethane	Vc DCM	0.05±1.34	1.82±1.77	>100
Kielmeyera membranacea	Dichloromethane	Km DCM	<0.8	4.70±1.84	>100
Vitex polygama	Crude Extract	Vp CE	78.23±1.97	>100	>100
	Hexane	Vp HEX	48.51±2.02	>100	>100
	Dichloromethane	Vp DCM	12.91±1.61	>100	>100
	Butanol	Vp BuOH	75.07±1.74	>100	>100

L-NMMA, a inhibitor of the inducible isoform of nitric oxide synthase (iNOS), at 20 µg/mL showed 95.15±7.33 % of inhibition on NO production; NO = nitric oxide; TNF-α = Tumor necrosis factor-alpha; SD = standard deviation; IC_{50} = the half maximal inhibitory concentration; CC_{50} = the 50% cytotoxic concentration.

showed concentration-dependent activity and reduced mycobacterial intracellular growth from 9.35×10^4 to 0.25×10^4 CFU/mL (97.53 ± 2.47%) at 0.8 µg/mL.

These results importantly enabled us to observe that the antimycobacterial activity was maintained, even when evaluated against intracellular growth. Considering that the antimycobacterial activity of extracts and fractions in infected macrophages has still been little discussed, this may be a relevant assay after initial screening.

These studies of infected macrophages may assist in identifying specific samples able to inhibit *M. tuberculosis* growth intracellularly, thus improving the ability to identify active compounds and excluding possible interferences from the permeability and conversion of the sample to the host cell, as well as the metabolism of the active compound before bacillus elimination (Sorrentino *et al*. 2015).

Our findings suggested that bioactivity resides in the dichloromethane fractions of *K. membranaceae* and *E. crotonoides*, therefore these two fractions were selected for chemical characterization by gas chromatography coupled with mass spectrometry (CG-MS).

GC-MS chromatogram of the Km DCM showed four peaks, from which one was predominant (Fig. 1) and was identified by comparing with the NIST library. The prevailing compound was a structure of pentacyclic triterpene (89.82%) eluted at 31.82 min (Tab. 4). Ec DCM showed 13 peaks, from which six were predominant (Fig. 2) and were identified using the NIST library (National Institute of Standards Technlogy). The six prevailing compounds were propanoic acid (7.93%) eluted at 4.27 min, hexadecanoic acid (6.22%) eluted at 17.96 min, oleic acid (13.55%) eluted at 19.60 min, octadecanoic acid (7.02%) eluted at 19.82 min, ricinoleic acid (36.10%) eluted at 21.36 min, and most likely a derivative of ricinoleic acid (6.10%) eluted at 23.43 min (Tab. 5).

Thus, the mass spectrometry analyses of dichloromethane fractions indicated the presence of terpenes and fatty acids. These results corroborate with other reports in the literature, which describe anti-mycobacterial activities for the same secondary metabolite classes (Jiménez-Arellanes *et al*. 2013; Luo *et al.* 2011; Carballeira 2008).

According to the references of this study, this is the first report with *in vitro* anti-mycobacterial and anti-inflammatory properties for the studied

Restinga species. The obtained results are partial scientific validations for the species upon known ethnopharmacological uses of their genus, highlighting the *K. membranaceae* and *E. crotonoides* species.

In summary, anti-mycobacterial activities observed for the species in this study indicate the presence of promising compounds. *K. membranaceae* and *E. crotonoides* were the most active species. We have successfully demonstrated

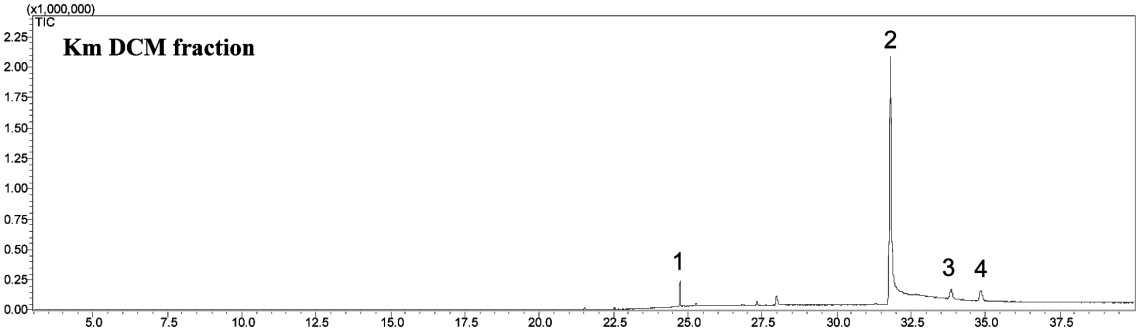

Figure 1 – Chromatographic profile of dichloromethane fraction from *Kielmeyera membranacea* obtained by gas chromatography coupled with mass spectrometer (GC-MS) after derivatization using N-methyl-trimethylsilyltrifluoroacetamide (MSTFA).

Table 4 – Chemical composition of dichloromethane fraction from *Kielmeyera membranacea* by gas chromatography coupled with mass spectrometer GC-MS.

Peak	t_R (min)	Area (% of total)	ID	Similarity (%)
1	24.73	3.26	Squalene	89
2	31.82	89.82	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6, 6a,6b,7,8,8a, 9,10,11,12,12a,14,14a,14b-octadecahydro-2H-iceno-3-one	88
3	33.85	3.01	Unidentified	-
4	34.85	3.91	Unidentified	-

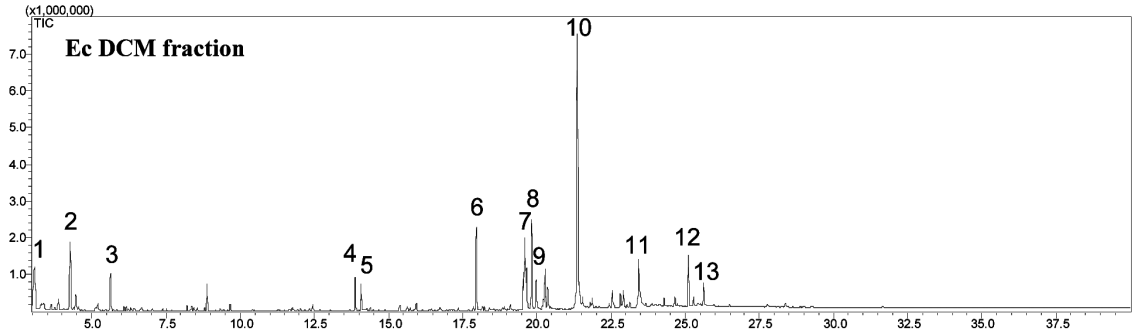

Figure 2 – Chromatographic profile of dichloromethane fraction from *Eremanthus crotonoides* obtained by gas chromatography coupled with mass spectrometer (GC-MS) after derivatization using N-methyl-trimethylsilyltrifluoroacetamide (MSTFA).

Table 5 – Chemical composition of dichloromethane fraction from *Eremanthus crotonoides* by gas chromatography coupled with mass spectrometer GC-MS.

Peak	t_R (min)	Area (% of total)	ID	Similarity (%)
1	3.07	6.10	Silanamine, N,1,1,1-tetramethyl-N-(Trimethylsilyl)	82
2	4.27	7.93	Unidentified	84
3	5.62	3.33	Propanoic acid, 2-[(trimethylsilyl)oxy]	91
4	13.88	2.60	Unidentified	-
5	14.08	1.99	Unidentified	-
6	17.96	6.22	Hexadecanoic acid, trimethylsilyl ester	91
7	19.60	13.55	Oleic acid, trimethylsilyl ester	89
8	19.82	7.02	Octadecanoic acid, trimethylsilyl ester	89
9	19.97	2.71	Unidentified	92
10	21.36	36.10	Ricinoleic acid, trimethylsiloxy, trimethylsilyl ester	96
11	23.43	6.10	Unidentified	85
12	25.11	4.43	Unidentified	-
13	25.62	1.92	Unidentified	-

that dichloromethane fractions of *K. membranaceae* and *E. crotonoides* contain anti-mycobacterial activity which can inhibit both avirulent *M. bovis* BCG and virulent *M. tuberculosis* H_{37}Rv strains. They also constitute viable sources for identifying anti-mycobacterial compounds which also present anti-inflammatory properties, and which may be potentially useful for treating severe TB cases.

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References

Amaral RR, Fernandes CP, Caramel OP, Tietbohl LA, Santos MG, Carvalho JC & Rocha L (2013) Essential oils from fruits with different colors and leaves of Neomitranthes obscura (DC.) N. Silveira: an endemic species from Brazilian Atlantic forest. Biomed Research International 2013: 723181.

Araujo MH, Silva ICV, Oliveira PF, Barreto ARR, Konno TUP, Esteves FA, Barth T, Aguiar FA, Lopes NP, Dermenjian RK, Guimarães DO, Leal ICR, Lasunskaia EB & Muzitano MF (2017) Biological activities and phytochemical profile of *Passiflora mucronata* from the Brazilian restinga. Revista Brasileira de Farmacognosia 27: 702-710.

Bussmann RW, Sharon D, Perez FA, Díaz DP, Ford T, Rasheed T, Barocio Y & Silva R (2008) Antibacterial activity of northern-peruvian medicinal plants. Arnaldoa 15: 127-148.

Carballeira NM (2008) New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. Progress in Lipid Research 47: 50-61.

Carneiro GRA, Silva AMS, Cavalcante RM, Padilha MC, Aquino Neto FR, Pereira HMG & Sardela VF (2018) Fast ephedrine quantification by gas chromatography mass spectrometry. Journal of the Brazilian Chemical Society 29: 2514-2521.

Cogliatti-Carvalho L, Freitas AFN, Rocha CFD & Van Sluys M (2001) Variation in structure and composition of Bromeliaceae at five zones of "restinga" in Jurubatiba National Park, Macaé, RJ. Revista Brasileira de Botânica 24: 1-9.

Coop BR & Pearce AN (2007) Natural product growth inhibitors of *Mycobacterium tuberculosis*. Natural Product Reports 24: 278-297.

Critchley JA, Young F, Orton L & Garner P (2013) Corticosteroids for prevention of mortality in people with tuberculosis: a systematic review and meta analysis. The Lancet Infectious Diseases 13: 223-237.

Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, Furin J, Nardell EA, London L, Lessem E, Theron G, van Helden P, Niemann S, Merker M, Dowdy D, Van Rie A, Siu GK, Pasipanodya JG,

Rodrigues C, Clark TG, Sirgel FA, Esmail A, Lin HH, Atre SR, Schaaf HS, Chang KC, Lange C, Nahid P, Udwadia ZF, Horsburgh CR Jr, Churchyard GJ, Menzies D, Hesseling AC, Nuermberger E, McIlleron H, Fennelly KP, Goemaere E, Jaramillo E, Low M, Jara CM, Padayatchi N & Warren RM (2017) The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respiratory Medicine 5: 291-360.

Garlanda C, Di Liberto D, Vecchi A, La Manna MP, Buracchi C, Caccamo N, Salerno A, Dieli F & Mantovani A (2007) Damping excessive inflammation and tissue damage in *Mycobacterium tuberculosis* infection by Toll IL-1 receptor 8/single Ig IL-1-related receptor, a negative regulator of IL-1/TLR signaling. Journal of Immunology 179: 3119-3125.

Gomez-Flores R, Gupta S, Tamez-Guerra R & Mehta RT (1995) Determination of MIC's for Mycobacterium avium-M. intracellulare complex in liquid medium by a colorimetric method. Journal of Clinical Microbiology 33: 1842-1846.

Griess P (1879) Bemerkungen zu der Abhandlung der HH: Weselsky und Benedikt 'Uber einige Azoverbindungen'. Berichte der Deutschen Chemischen Gesellschaft 12: 426-428.

Gu JQ, Wang Y, Franzblau SG, Montenegro G, Yang D & Timmermann BN (2004) Antitubercular constituents of *Valeriana laxiflora*. Planta Medica 70: 509-514.

Gupta VK, Shukla C, Bisht GR, Saikia D, Kumar S & Thakur RL (2011) Detection of anti-tuberculosis activity in some folklore plants by radiometric BACTEC assay. Letters in Applied Microbiology 52: 33-40.

Habtamu A & Melaku Y (2018) Antibacterial and antioxidant compounds from the flower extracts of *Vernonia amygdalina*. Advances in Pharmacological Sciences 2018: 4083736.

Harvey AL, Clark RL, Mackay SP & Johnston BF (2010) Current strategies for drug discovery through natural products. Expert Opinion on Drug Discovery 5: 559-568.

Jiménez-Arellanes A, Luna-Herrera J, Cornejo-Garrido J, López-García S, Castro-Mussot ME, Meckes-Fischer M, Mata-Espinosa D, Marquina B, Torres J & Hernández-Pando R (2013) Ursolic and oleanolic acids as antimicrobial and immunomodulatory compounds for tuberculosis treatment. BMC Complementary and Alternative Medicine 13: 258-268.

Lasunskaia E, Ribeiro SC, Manicheva O, Gomes LL, Suffys PN, Mokrousov I, Ferrazoli L, Andrade MR, Kritski A, Otten T, Kipnis TL, Silva WD, Vishnevsky B, Oliveira MM, Gomes HM, Baptista IF & Narvskaya O (2010) Emerging multidrug resistant *Mycobacterium tuberculosis* strains of the Beijing genotype circulating in Russia express a pattern of biological properties associated with enhanced virulence. Microbes and Infection 12: 467-475.

Kannathasan K, Senthilkumar A & Venkatesalu V (2011) In vitro antibacterial potential of some Vitex species against human pathogenic bacteria. Asian Pacific Journal of Tropical Medicine 4: 645-648.

Koul A, Herget T, Klebl B & Ullrich A (2004) Interplay between mycobacteria and host signalling pathways. Nature Reviews Microbiology 2: 189-202.

Kuo LY, Tseng PY, Lin YC, Liaw CC, Zhang LJ, Tsai KC, Lin ZH, Ho HO & Kuo YH (2018) New hirsutinolide-type sesquiterpenoids from *Vernonia cinerea* inhibit nitric oxide production in LPS-stimulated RAW264.7 cells. Planta Medica 84: 1348-1354.

Luo X, Pires D, Aínsa JA, Gracia B, Mulhovo S, Duarte A, Anes E & Ferreira MJ (2011) Antimycobacterial evaluation and preliminary phytochemical investigation of selected medicinal plants traditionally used in Mozambique. Journal of Ethnopharmacology 137: 114-120.

Machado FLS, Ventura TL, Gestinari LM, Cassano V, Resende JA, Kaiser CR, Lasunskaia EB, Muzitano MF & Soares AR (2014) Sesquiterpenes from the Brazilian red alga *Laurencia dendroidea* J. Agardh. Molecules 19: 3181-3192.

Mahairas GG, Sabo PJ, Hickey MJ, Singh DC & Stover CKJ (1996) Molecular analysis of genetic differences between Mycobacterium bovis BCG and virulent M. bovis. Journal Bacteriology 178: 1274-1282.

Melo MS, Brito RG, Santos PL, Nogueira PC, Moraes VR, Matos MC, Ferro JN, Barreto EO, Lucca Junior W, Botelho MA & Quintans Junior LJ (2014) Involvement of cerebral nervous system areas and cytokines on antihyperalgesic and anti-inflammatory activities of *Kielmeyera rugosa* Choisy (Calophyllaceae) in rodents. Phytotherapy Research 28: 1806-1815.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and citotoxicity assay. Journal of Immunology Methods 65: 55-63.

Muzitano MF, Cruz EA, Almeida AP, Silva AS, Kaiser CR, Guette C, Rossi-Bergmann B & Costa SS (2006) Quercitrin: an antileishmanial flavonoid glycoside from Kalanchoe pinnata. Planta Medica 72: 81-83.

Okunade AL, Elvin-Lewis MP & Lewis WH (2004) Natural antimycobacterial metabolites: current status. Phytochemistry 65: 1017-1032.

Orme I (2001) Tuberculosis drug screening program. Search for new drugs for treatment of tuberculosis. Antimicrobial Agents Chemotherapy 45: 1943-1946.

Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, Ginsberg A, Swaminathan S,

Spigelman M, Getahun H, Menzies D & Raviglione M (2016) Tuberculosis. Nature Reviews Disease Primers 2: 16076.

Pinheiro L, Nakamura CV, Dias Filho BP, Ferreira AG, Young MCM & Cortez AG (2003) Antibacterial xanthones from *Kielmeyera variabilis* mart. (Clusiaceae). Memórias do Instituto Oswaldo Cruz 98: 549-552.

Princely NS, Basha JS, Kirubakaran J & Dhanaraju MD (2013) Preliminary phytochemical screening and antimicrobial activity of aerial parts of *Stachytarpheta indica* L. (Vahl.). Medicinal Plants - International Journal of Phytomedicines and Related Industries 5: 96-101.

Roumy V, Fabre N, Portet B, Bourdy G, Acebey L, Vigor C, Valentin A & Moulis C (2009) Four anti-protozoal and anti-bacterial compounds from *Tapirira guianensis*. Phytochemistry 70: 305-311.

Salomon CE & Schmidt LE (2012) Natural products as leads for tuberculosis drug development. Current Topics in Medicinal Chemistry 12: 735-765.

Santos FM, Malafaia CA, Simas DLR, Paulino AB, Muzitano MF, Simas NK, Cruz Da-Rocha EA, Amaral ACF & Leal ICR (2019) Phenolic compounds from *Tocoyena bullata* mart (Rubiaceae) with inhibitory activity in mast cells degranulation. Natural Product Research 19: 1-4.

Santos MG, Fevereiro PCA, Reis GL & Barcelos JI (2010) Recursos vegetais da Restinga de Carapebus, Rio de Janeiro, Brasil. Journal of Neotropical Biology 6: 35-54.

Silva JB, Bessa ME, Santos Mayorga OA, Andrade VT, Costa YFG, Freitas Mendes R, Pires Ferreira AL, Scio E & Alves MS (2018) A promising antibiotic, synergistic and antibiofilm effects of *Vernonia condensata* Baker (Asteraceae) on Staphylococcus aureus. Microbial Pathogenesis 123: 385-392.

Sorrentino F, Gonzalez del Rio R, Zheng X, Matilla JP, Gomez PT, Hoyos MM, Herran MEP, Losana AM & Av-Gay Y (2015) Development of an intracellular screen for new compounds able to inhibit *Mycobacterium tuberculosis* growth in human macrophages. Antimicrobial Agents Chemotherapy 60: 640-645.

Sousa ZL, Oliveira FF, Conceição AO, Silva LAM, Rossi MH, Santos JS & Andrioli JL (2012) Biological activities of extracts from *Chenopodium ambrosioides* Lineu and Kielmeyera neglecta Saddi. Annals of Clinical Microbiology and Antimicrobials 11: 1-7.

Sreelatha R, Murali Mohan CH, Ashok Phani Kiran K & Sudesh Kumar E (2014) *In vitro* antimicrobial activity of different parts of *Stachytarpheta urticifolia* (Salisb) Sims. International Journal of Pharmacy and Pharmaceutical Sciences 6: 340-343.

Toledo CE, Britta EA, Ceole LF, Silva ER, Mello JC, Dias Filho BP, Nakamura CV & Ueda-Nakamura T (2011) Antimicrobial and cytotoxic activities of medicinal plants of the Brazilian cerrado, using Brazilian cachaça as extractor liquid. Journal of Ethnopharmacology 133: 420-425.

Tosun F, Kizilay CA, Sener B, Vural M & Palittapongarnpim P (2004) Antimycobacterial screening of some Turkish plants. Journal of Ethnopharmacology 95: 273-275.

Ventura TLB, Calixto SD, Abrahim-Vieira BA, Souza AM, Mello MV, Rodrigues CR, Miranda LSM, Souza ROA, Leal ICR, Lasunskaia EB & Muzitano MF (2015a) Antimycobacterial and anti-inflammatory activities of substituted chalcones focusing on an anti-tuberculosis dual treatment approach. Molecules 20: 8072-8093.

Ventura TLB, Machado FLS, Araujo MH, Gestinari LMS, Kaiser CR, Esteves FA, Lasunskaia EB, Soares AR & Muzitano MF (2015b) Nitric oxide production inhibition and anti-mycobacterial activity of extracts and halogenated sesquiterpenes from the brazilian red alga *Laurencia dendroidea* J. Agardh. Pharmacognosy Magazine 11: S611-618.

Ventura TLB, Silva DRC, Lasunskaia EB, Maria EJ, Muzitano MF & Oliveira RR (2015c) Coumarine analogues with antimycobacterial and immunomodulatory activity. Current Bioactive Compounds 11: 109-115.

WHO (2019) Global Tuberculosis Report. World Health Organization, Geneva. 265p.

Yaseen R, Branitzki-Heinemann K, Moubasher H, Setzer WN, Naim HY & von Köckritz-Blickwede M (2017) In vitro testing of crude natural plant extracts from Costa Rica for their ability to boost innate immune cells against Staphylococcus aureus. Biomedicines 5: E40.

Zhang Q, Jiang X, He W, Wei K, Sun J, Qin X, Zheng Y & Jiang X (2017) MCL Plays an anti-inflammatory role in Mycobacterium tuberculosis-induced immune response by inhibiting NF-κB and NLRP3 inflammasome activation. Mediators of Inflammation 2017: 2432904.

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