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Medicinal plants from open-air markets in the State of Rio de Janeiro, Brazil as a potential source of new antimycobacterial agents

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ABSTRACT

Ethnopharmacological relevance: Several medicinal plants are traditionally traded in open-air markets in Rio de Janeiro State (Brazil) to treat tuberculosis (TB) and related symptoms.**Aims of the study:** Conduct a survey in the open-air markets of 20 cities of Rio de Janeiro State to find medicinal plants that are popularly used to treat tuberculosis and other related diseases and assess their *in vitro* antimycobacterial activity.**Materials and methods:** We used direct observation and semi-structured interviews and asked herbalists to list species (free listing) in order to gather data about the plant species most commonly used for lung problems. We calculated a Saliency Index and acquired two species of “erva-de-passarinho” (mistletoe), *Struthanthus marginatus* and *Struthanthus concinnus* (Loranthaceae), commonly used to treat tuberculosis for a bioassay-guided isolation of the antimycobacterial active principles. Extracts, fractions and isolated compounds of both species were assayed *in vitro* against susceptible (H₃₇Rv) and rifampicin-resistant (ATCC 35338) *Mycobacterium tuberculosis* strains.**Results:** From the interviews, we generated a list of 36 plant species belonging to 12 families. The mistletoes *Struthanthus marginatus* and *Struthanthus concinnus* showed high Saliency Index values among plants used to treat tuberculosis. Bioassay-guided fractionation of hexane extracts from both species led to the isolation and/or identification of steroids and terpenoids. The Minimum Inhibitory Concentration (MIC) of the extracts and isolated compounds ranged from 25 to 200 µg/mL. Some of the isolated compounds have been previously assayed against *Mycobacterium tuberculosis*, others are reported here for the first time (obtusifoliol: MIC H₃₇Rv 50 µg/mL, MIC ATCC 35338 12.5 µg/mL; 3-*O*-*n*-acil-lup-20(29)-en-3β,7β,15α-triol: MIC H₃₇Rv 200 µg/mL, MIC ATCC 35338 100 µg/mL).**Conclusions:** This study demonstrated the importance of ethnobotanical surveys in markets as a source for new drugs and also for scientific validation of folk medicine.

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1. Introduction

Ethnobotany and ethnopharmacology have been the primary approaches used by scientists worldwide to select medicinal plants that may result in new drugs for medical and pharmaceutical interests (Albuquerque and Hanazaki, 2006). People everywhere use plants to fulfil their biological, cultural and economic needs. When removed from daily contact with the sources of plant materials, they depend on an organised exchange structure such as a market to obtain these plants (Bye and Linares, 1983; Balick

and Lee, 2001; Silva, 2008). The biocultural diversity utilized in herbal medicine is not restricted to dispersed and marginal communities; it is also very much a part of metropolitan areas (Pieroni and Vandebroek, 2007). The botanical knowledge of urban populations is complex, with a set of knowledge and beliefs about vegetal elements that interacts within the same pluricultural scope (Pochettino et al., 2012). Therefore, open-air markets are a good source of information for ethnobotanical research, including the use of many medicinal and ornamental plants, foods and other products that have regional value (Martin, 2000; Nguyen, 2005; Albuquerque et al., 2007).

Open-air markets can be described as urban places with an intense interaction among people from different socio-economic and cultural groups, and plant use serves to link and share knowledge from

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different regions and origins (Bye and Linares, 1983; Albuquerque et al., 2007; Silva, 2008). Local markets unite, concentrate, maintain, and diffuse empirical knowledge about plant and animal resources (Albuquerque et al., 2007; Monteiro et al., 2010).

Ethnobotanical studies focusing on herbalists in traditional markets are still scarce (Jain, 2000; Albuquerque et al., 2007). In Brazil there have been a handful of ethnobotanical studies in open-air markets (Berg, 1984; Silva et al., 2001; Almeida and Albuquerque, 2002; Pinto and Da, Maduro, 2003; Albuquerque et al., 2007), and only a few studies in open-air markets in the state of Rio de Janeiro (Santos and Silvestre, 2000; Stalcup, 2000; Parente and Rosa, 2001; Azevedo and Silva, 2006; Maioli-Azevedo and Fonseca-Kruel, 2007; Silva, 2008; Leitão, 2009a; Leitão et al., 2009b) (Fig. 1).

Plant species and natural products serve as a rich source of many novel biologically active compounds and can be a source of medicines and new drugs, including new anti-tubercular agents (Asres et al., 2001; Heinrich and Gibbons, 2001; Copp, 2003; Leitão et al., 2006; Gupta et al., 2010; Mohamad et al., 2011; Oliveira et al., 2011). Tuberculosis (TB) is an infectious disease caused mainly by *Mycobacterium tuberculosis*. According to the World Health Organization (WHO), nearly one-third of the global population is infected with *Mycobacterium tuberculosis*, more than 8 million people still develop active TB each year, and almost two million people die from it (WHO, 2011). Over 90% of global TB cases and deaths occur in the developing world (Lall and Meyen, 1999; WHO, 2011). In Brazil, there are an estimated 72,000 new cases of TB and 4700 deaths each year (Brazil, 2011). Brazil is among the 22 countries with the highest prevalences of TB in the world (Brazil, 2004), and in 2003, the Ministry of Health of Brazil defined TB control as a national priority (Brazil, 2011). In the State of Rio de Janeiro, the incidence of the disease (65.1 cases per 100,000 inhabitants in 2012) is among the highest rates registered in

the country and almost twice the national average (36.1 cases per 100,000 inhabitants in 2012) (SINAN, 2013). In Brazil, anti-TB medication is freely distributed, but there are many cases of noncompliance with treatment, mainly because of the long duration required for successful chemotherapy (FUNASA, 2002). Furthermore, drug resistance, mainly multidrug resistance (MDR), although still present at a low level in Brazil, has increased in recent years. This makes the search for new anti-tubercular agents an important task.

As part of a broader project searching for new anti-tubercular agents from natural sources, a preliminary study from our group in two open-air markets in the mountain region of the State of Rio de Janeiro was conducted. Plants that were commercialised to combat TB and other associated diseases were recorded and two “ervas-de-passarinho” (mistletoes) (*Struthanthus concinnus* and *Struthanthus marginatus*) were distinctive among other plants for the treatment of respiratory ailments (Leitão, 2009a). These results prompted us to conduct a larger study to inventory the medicinal plant species used to treat TB and related diseases that were traded in open-air markets in 20 cities throughout the state. Interestingly, these same mistletoes were the most important species and therefore selected for *in vitro* antimycobacterial bioassay guided fractionation.

2. Materials and methods

2.1. Study area

The present study was conducted in open-air markets of the state of Rio de Janeiro, Brazil, between March 2009 and August 2010 (Fig. 1). Rio de Janeiro has a total area of 43,780.157 km² and a population of 15,989,929 inhabitants (365.23 inhabitants/km²) distributed in 92 municipalities (IBGE, 2011). Because it was

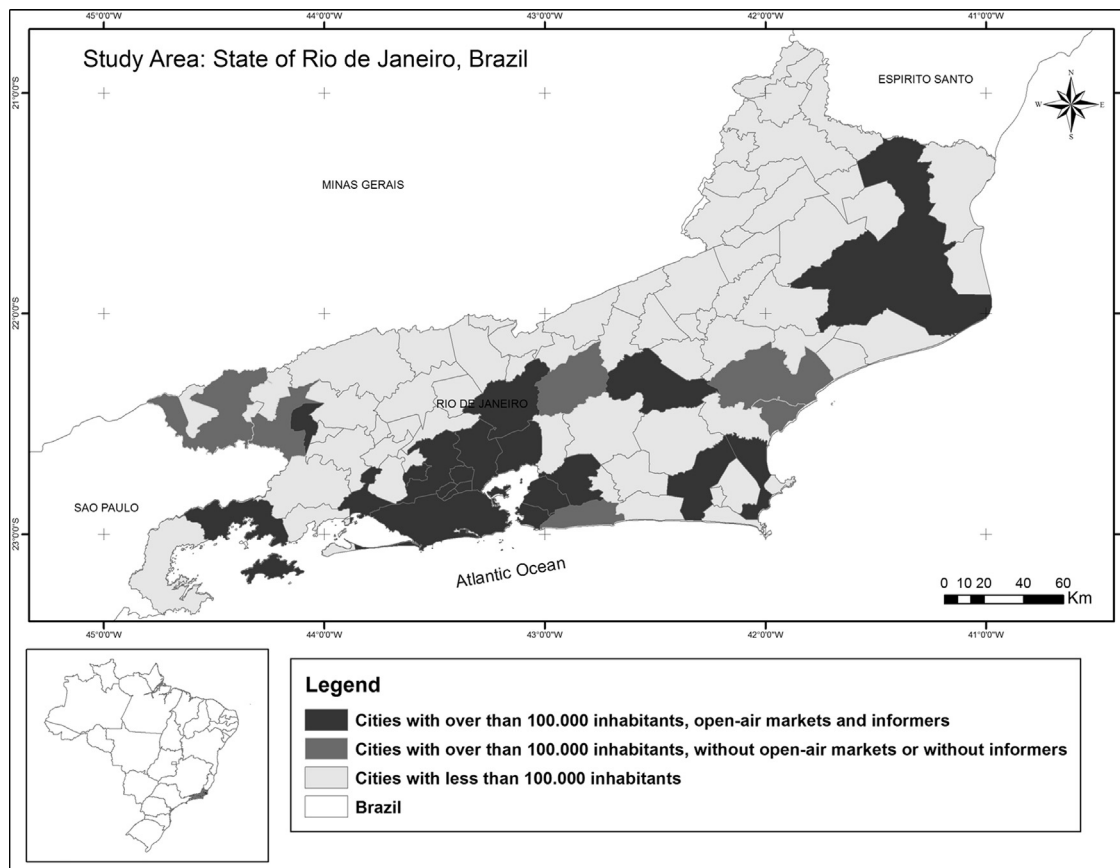


Fig. 1. Map of the study area in the State of Rio de Janeiro, Brazil.

difficult to visit them all, we selected locations based on a criterion of the Brazilian Ministry of Health, which establishes that the number of inhabitants in a city must be equal to or greater than 100,000 and the incidence rate of TB cases must be over 47 per 100,000 inhabitants to prioritise locations to combat TB (Brazil, 2004). Because of this criterion and because this is an urban ethnobotanical study, only municipalities with more than 100,000 inhabitants were selected (Fig. 1).

2.2. Human aspects

Of the 26 cities with over 100,000 inhabitants in Rio de Janeiro, only 20 had open-air markets that sold medicinal plants (Fig. 1). These markets were visited to identify and contact informants in the tents selling medicinal plants who could be considered “herbalists” or “specialists”. The informants were identified using the “snow-ball” method (Bernard, 2011). Sampling was non-random under the assumption that local herbalists would give more specific information regarding medicinal plants (Bailey, 1994; Alexiades, 1996; Albuquerque and Lucena, 2004). Thirty herbalists were interviewed in the selected municipalities.

We used direct observation and semi-structured interviews and asked herbalists to list species (free listing) in order to determine the most commercialised species (Weller and Kimball Romney, 1988; Alexiades, 1996; Bernard, 2011). Ethical approval for this study was obtained from the Instituto de Estudos em Saúde Coletiva (IESC/UFRJ) under registration number N°27/2010.

A specific questionnaire was developed to focus on plants sold to be helpful for “lung problems”, “cough” and “tuberculosis”, designations that may indicate possible antimycobacterial activity. The Saliency Index was calculated (Quinlan, 2005) for free listed plants using the Anthropac 4.0 software program (Atick, Analytic Technologies, MA, USA), (Borgatti, 1996; Araújo et al., 2008).

2.3. Plant materials

The plants indicated for the treatment of lung problems, cough and tuberculosis by each informant were bought and identified with the aid of specialists from the Jardim Botânico do Rio de Janeiro (RB), following APGIII (Angiosperm Phylogeny Website, 2008; Souza and Lorenzi, 2008; Judd et al., 2009). Herbarium voucher specimens are preserved in the Herbarium of the Jardim Botânico do Rio de Janeiro (RB) and at Universidade Federal do Rio de Janeiro (RFA) (Table 1).

The selection of plants was based on the experience of informants to treat tuberculosis. Two species of “erva-de-passarinho” (mistletoe), *Struthanthus marginatus* (Loranthaceae; voucher specimens RB 468 941 and RFA 34490) and *Struthanthus concinnus* (Loranthaceae; voucher specimens RB 469119 and RFA 34484), were indicated to treat tuberculosis by informants and were acquired for phytochemical analysis and biological assays.

2.4. Preparation of plant extracts

The aerial parts of *Struthanthus marginatus* and the leaves of *Struthanthus concinnus* were dried and extracted exhaustively with pure ethanol, which was evaporated under reduced pressure. The residue was suspended in water and then partitioned successively with water and hexane, dichloromethane, ethyl acetate and *n*-butanol as described elsewhere (Leitão et al., 2013). Aqueous extracts of both species were also prepared following the instructions given by the informants to prepare tea (the traditional method) as follows: five leaves of *Struthanthus marginatus* in a glass of water (5 g/250 mL) and 8 leaves of *Struthanthus concinnus* in 500 mL of water (2 g/500 mL). The teas were then freeze-dried, producing a pale brown

powder. The yields were 8.89% (*Struthanthus marginatus*) and 11.02% (*Struthanthus concinnus*).

2.5. Bioassay-guided fractionation and the isolation of pure compounds from active hexane extracts.

The hexane extracts of both plants (*Struthanthus marginatus* and *Struthanthus concinnus*) were submitted to fractionation by liquid chromatography on silica gel to produce 33 fractions, which were assigned the labels SMH-1 to SMH-33 (*Struthanthus marginatus*), and 62 fractions, labelled SCH-1 to SCH-62 (*Struthanthus concinnus*), according to their chromatographic profile, as described previously (Leitão et al., 2013).

2.6. Anti-tuberculosis activity assay.

The crude extracts, fractions and isolated compounds from *Struthanthus marginatus* and *Struthanthus concinnus* were screened against *Mycobacterium tuberculosis* strains H₃₇Rv (sensitive) and ATCC 35338 (resistant to rifampicin) by the microdilution method using resazurin as an indicator of cell viability, as previously reported (Palomino et al., 2002; Castellar et al., 2011; Oliveira et al., 2011). In the initial screening, a fixed concentration of 200 µg/mL was used. For the active samples and positive control, a minimum inhibitory concentration (MIC) was determined. The assays were conducted based on the literature (Palomino et al., 2002) with minor modifications.

3. Results and discussion

3.1. Open-air markets and ethnobotanical survey

Due to the high incidence of TB in the State of Rio de Janeiro, especially in cities with large numbers of inhabitants, 26 municipalities with over 100,000 inhabitants were chosen for this study based on criteria used by the Brazilian Ministry of Health (Section 2.1). The open-air markets at 22 municipalities were visited, but only 20 of them had medicinal plants for sale (Figs. 1 and 2). Each municipality studied, except Mesquita, is among the 28 government priority sites for TB (Brazil, 2004).

In the 20 municipalities where open-air markets and commercialisation of medicinal plants were found, 30 sellers were interviewed, 15 of each gender. Their ages varied between 20 and 79 years old, and the average age was 54 (20–50: 33.33%; 51–79: 66.67%). The sellers described different ways of learning about the plants: most of them (16) acquired their knowledge about medicinal plants from their relatives or acquaintances (grandparents, parents, spouses, in-laws, friends or other sellers). This indicates that, despite the alterations in the market system, vertical transmission between people persists. In Uganda, the knowledge of traditional medicine practitioners, including how to treat TB, is mainly acquired from parents and other relatives (Tabuti et al., 2010). In Brazil, the sellers teach consumers, relatives and acquaintances about plants. In the present study, plants were mainly purchased, although they were also collected or cultivated. Specific plants to treat TB were indicated by 43.3% of the sellers.

During the interviews, the informants listed 36 species for the treatment of cough, lung problems and tuberculosis. Those species were collected and distributed in 12 botanical families (Table 1).

The most common family was Asteraceae (14 spp.), followed by Lamiaceae (4 spp.) (Table 1). Both families tend to be the most important in ethnobotanical studies of markets (Stalcup, 2000; Parente and Rosa, 2001; Azevedo and Silva, 2006; Maioli-Azevedo and Fonseca-Kruel, 2007; Silva, 2008; Monteiro et al., 2010) and other ethnobotanical studies (Gazzaneo et al., 2005; Sá IM, 2007;

Table 1

A list of the plants indicated for the treatment of “lung problems”, “cough” and “tuberculosis”, from the open-air markets in the state of Rio de Janeiro, Brazil.

Family (APGIII) Scientific name	Popular name	Form of preparation				Together with other plants	Voucher
		Tea	Syrup	Juice/ use of blender	Other		
Amaranthaceae <i>Chenopodium ambrosioides</i> L.	(erva de) Santa Maria, mastruz, mentruz	X	X	X	X	X	RB454891, RFA37469
Asphodelaceae <i>Aloe arborescens</i> Mill.	Babosa		X	X		X	RFA37471
Asteraceae <i>Artemisia</i> sp.	Cânfora	X				X	RFA37478
<i>Bidens pilosa</i> L.	Picão preto	X				X	RFA37475
<i>Elephantopus mollis</i> Kunth.	Erva grossa	X					RB 468912
<i>Heterocondylus alatus</i> (Vell.) R.M.King and H.Rob.	Assa peixe	X	X			X	RFA37477
<i>Hypochaeris brasiliensis</i> (Less.) Hook. and Arn.	Cardo santo	X		X		X	RB454870
<i>Mikania laevigata</i> Sch. Bip. ex Baker	Guapo, guaco, guacro	X	X	X		X	RB454877, RFA37473
<i>Solidago chilensis</i> Meyen	Arnica da horta			X			RFA37474
<i>Vernonia phaeoneura</i> Toledo	Assa peixe		X				RB454884
<i>Vernonia polyanthes</i> Less.	Assa peixe	X	X	X		X	RFA37467
<i>Vernonia westiniana</i> Less.	Assa peixe		X				RFA37476
<i>Vernonia</i> sp.	Assa peixe	X	X				
Undet. 1	Assa peixe	X		X		X	
Undet. 2	Assa peixe		X	X		X	
Undet. 3	Assa peixe		X	X		X	
Brassicaceae <i>Nasturtium officinale</i> R. Br	Agrião		X	X		X	RFA37468
Crassulaceae <i>Kalanchoe brasiliensis</i> Cambess.	Saião	X	X	X		X	RB454893, RFA37479
Fabaceae <i>Hymenaea</i> sp.	Jatobá	X	X			X	RFA37472
Lamiaceae <i>Mentha pulegium</i> L.	Poejo	X	X	X		X	RFA36982, RFA36983
<i>Mentha x piperita</i> L.	Hortelã	X				X	RFA36985
<i>Ocimum campechianum</i> Mill.	Alfavaca					X	RFA36984
<i>Ocimum gratissimum</i> L.	Alfavaca	X	X	X		X	RB454906
Loranthaceae <i>Struthanthus concinnus</i> Mart.	Erva de passarinho		X			X	RB454917, RB469119
<i>Struthanthus marginatus</i> (Desr.) G. Don	Erva de passarinho	X	X	X		X	RFA37480, RFA37481
Malvaceae <i>Malva</i> sp.	Malva		X			X	
Myrtaceae <i>Eugenia uniflora</i> L.	Pitanga	X					RFA37482
Poaceae <i>Cymbopogon citratus</i> (DC.) Stapf	Cidreira ou capim-limão	X	X	X		X	RB 454937
Rutaceae <i>Citrus aurantium</i> L.	Laranja da terra						RFA37466
Unidentified barks	Suma	X	X			X	
	Aroeira	X					
	Cajú roxo	X					
	Pulmonaria	X	X			X	
	Angico	X	X			X	
	Jequitibá	X	X			X	
	Casca sagrada	X		X		X	
		26	24	17		2	28

Undet—Unidentified.

Tabuti et al., 2010). Asteraceae and Lamiaceae are distributed in cosmopolitan areas and known worldwide as edible, medicinal aromatic plants, which explains their prevalence in ethnobotanical work (Souza and Lorenzi, 2008; Judd et al., 2009; Tabuti et al., 2010). Asteraceae is the biggest family of vascular plants, with 23,000 species (Judd et al., 2009). The popularity of Asteraceae is believed to be due to the large diversity of bioactive components of its family members (Tabuti et al., 2010). The Lamiaceae family has circa 6800 species (Judd et al., 2009), rich in aromatic oils, which are appreciated for their therapeutic values (Tabuti et al.,

2010). These families are well represented in Brazil; Asteraceae with approximately 2000 species and Lamiaceae with 350 species (Souza and Lorenzi, 2008).

In this study, the most frequently cited form of medicinal plant preparation was tea (infusion or decoction) (26 species, 72.2%), followed by syrup (24 species, 66.7%) and juice (use of blender) (17 species, 47.2%). However, for many plant species, more than one type of preparation was reported. Syrups may be used more frequently for these plants because they are for cough and lung problems, for which syrups are customary or accepted, even in



Fig. 2. View of the medicinal plants sold at the open-air market of Petrópolis, Rio de Janeiro, Brazil.

non-traditional medicine. Tea is a widespread form of preparation in Brazil (Stalcup, 2000; Parente and Rosa, 2001; Gazzaneo et al., 2005; Silva, 2008). The common sale and consumption of drugs *in natura* prompted the government to issue a resolution (RDC 10) establishing quality criteria for the marketing of herbal drugs for infusions and decoctions (Brazil, 2010).

Another interesting result from our work shows that 36 species (77.8%) were used together with other plants (in mixtures) (Table 1). It is possible that interactions among different species in mixtures improve the therapeutic effects and attenuate the toxicity or adverse effects of some plants (Bruschi et al., 2011).

3.2. Saliency

The Saliency Index has been used extensively for analysing species mentioned by local specialists in a free-listing exercise (Araújo et al., 2008; Silva, 2008; Leitão, 2009a) because it considers the order in which species are listed and also the number of informants that mentioned each plant. The informants tend to first list the species that are more important, and the best-known plants are listed most frequently. Therefore, it is possible to confirm that the first plants on the list are the most important (Silva, 2008).

The questionnaire we designed to reveal species with possible antimycobacterial activity asked for free-lists, from which the saliency was calculated (Quinlan, 2005). The ethnospesies with the highest values that were prescribed for cough and lung problems were “assa peixe”, “guaco”, “saião” and “erva-de-passarinho” (Table 2). The term “ethnospesies” is used to designate the biological entities recognised by the informants (Albuquerque et al., 2011). An ethnospesies may or may not exactly correspond to a botanical species; two or more species may have the same popular name, or more than one popular name may correspond to the same taxon (Sá IM, 2007; Albuquerque et al., 2011; Bruschi et al., 2011).

The ethnospesies “assa peixe” was the most prominent plant. Purchased samples of “assa peixe” represented five different botanical species from the Asteraceae family: *Heterocondylus alatus* (Vell.) R.M. King and H. Rob., *Vernonia phaeoneura* Toledo, *Vernonia polyanthes* Vell., *Vernonia westiniana* Vell., and *Vernonia* sp. Some samples could not be identified (Table 1). “Assa peixe” is the popular name of *Vernonia* species, such as *Vernonia amygdalina*, *Vernonia westiniana*, *Vernonia hilariana*, *Vernonia grandiflora*, *Vernonia ferrugininea*, *Vernonia beyrichii*, *Vernonia lindbergii* and *Vernonia scorpioide* (Saad et al., 2009). In folk medicine, species of

Table 2

The first ten values in the calculation of Saliency (Quinlan, 2005) by ethnospesies that were indicated for the treatment of “lung problems” and “cough” (left) and specifically for treatment of “tuberculosis” (right) by interviewees in open-air markets in the state of Rio de Janeiro, Brazil.

Popular name	Saliency (“lung” and “cough”)	Popular name	Saliency (“TB”)
Assa peixe	0.607	Erva de passarinho	0.289
Guaco	0.475	Assa peixe	0.281
Saião	0.347	Mastruz or Santa Maria	0.237
Erva de passarinho	0.346	Saião	0.140
Mastruz or Santa Maria	0.257	Eucalipto	0.079
Poejo	0.218	Jatobá	0.079
Agrião	0.089	Angico	0.070
Jatobá	0.071	Erva grossa	0.061
Erva grossa	0.063	Cardo santo	0.053
Alfavaca	0.049	Cavalinha	0.053
		Espinheira santa	0.053
		Picão preto	0.053
		Pulmonaria	0.053

Obs. Some plants were not found to collect.

the genus *Vernonia* (Asteraceae) are used to treat many diseases worldwide (Iwalewa et al., 2003; Rauh et al., 2011). In contrast, the “guaco” and its variations such as “guapo” or “guacro” were also distinctive on the sellers’ lists, and every sample was identified as *Mikania laevigata* Sch. Bip. ex Baker (Asteraceae).

When we analysed the plants cited specifically to treat tuberculosis (Table 2), we found that “erva-de-passarinho” was the most commonly named plant. Samples of “erva-de-passarinho” were identified as two different species: *Struthanthus concinnus* Mart. and *Struthanthus marginatus* (Desr.) G. Don. (Loranthaceae). These plants are hemiparasites (mistletoes) and have a substance in the seeds that can fix them to the host plant after ingestion and excretion by birds (Reif de Paula CH, 2004). Mistletoes of the *Struthanthus* genus are used for treating many diseases in folk medicine, some related to the respiratory tract. In the state of Maranhão, northern Brazil, *Struthanthus marginatus* is recommended for stomach ailments and its gastroprotective activity has been observed *in vivo* with no detected signs of toxicity (Freire et al., 2011).

The most commonly indicated plant species should be examined in future biological assays because extracts of plant species from a wide range of families have been shown to have significant *in vitro* antimycobacterial activity (Newton et al., 2000) and because they serve as a rich source of many novel biologically active compounds (Gautam et al., 2007; Heinrich and Gibbons, 2001).

3.3. Literature scores for the antimycobacterial activity of plants cited in the ethnobotanical survey

Literature searches regarding the plants found in this study showed that some of them have already been tested against *Mycobacterium tuberculosis* and have presented antimicrobial activity. The bibliographic survey was performed using articles and reviews on antimycobacterial activity in natural products (Mitscher and Baker, 1998; Lall and Meyen, 1999; Newton et al., 2000; Copp, 2003; Okunade et al., 2004; Pauli et al., 2005; Cos et al., 2006; Jachak and Jain, 2006; Gautam et al., 2007; Molina-Salinas et al., 2006, 2007; Tabuti et al., 2010; Oliveira et al., 2011). Among other species listed in the present study, only three additional species were mentioned in the resources we used:

Table 3
Bibliographic survey of research on antimycobacterial activity for species sold in open-air markets in the state of Rio de Janeiro.

Botanic family	Species	Extract	Antimycobacterial activity	Used in folk medicine	Place where is used in folk medicine	References
Amaranthaceae	<i>Chenopodium ambrosioides</i>	Acetone	0.5 mg/mL (sensitive strain H37Rv) and 0.1 mg/mL (isoniazid and rifampin resistant strain)	Cough and for chest pain	South African	Lall and Meyen (1999)
Amaranthaceae	<i>Chenopodium ambrosioides</i>	Essential oil		Cough, pulmonary complaints	India	Gautam et al. (2007)
Amaranthaceae	<i>Chenopodium ambrosioides</i>			Cough, pneumonia, TB	Brazil	Oliveira et al. (2011)
Asteraceae	<i>Bidens pilosa</i>	Ethanol	100 µg/mL		Ruanda	Newton et al. (2000)
Asteraceae	<i>Bidens pilosa</i>	Ethanol	100 µg/mL	Indicated to treat pulmonary diseases	India	Gautam et al. (2007)
Asteraceae	<i>Bidens pilosa</i>			Traditional medicine practitioners for the treatment of TB and allied diseases	Uganda	Tabuti et al. (2010)
Fabaceae	<i>Hymenaea</i> sp. (species: <i>Hymenaea courbaril</i> L.)	Ethanol	Not active	Cough, pneumonia, TB	Brazil	Oliveira et al. (2011)
Lamiaceae	<i>Mentha piperita</i> L.	Methanol	> 500 µg/mL against <i>Mycobacterium avium</i> and <i>Mycobacterium smegmatis</i>	It is used to treat cough and chest complaints (Newton et al., 2000)	India	Gautam et al. (2007)

Chenopodium ambrosioides, *Vernonia polyanthes*, *Bidens pilosa*, *Hymenaea* sp. and *Mentha piperita* (Table 3).

Chenopodium ambrosioides is used to treat TB-related respiratory disorders, such as asthma, cold, cough, flu and pulmonary complaints, in the South African, Ayurvedic and Mexican systems of medicine (Lall and Meyen, 1999; Molina-Salinas et al., 2006; Gautam et al., 2007). The essential oil of *Chenopodium ambrosioides* inhibits the growth of *Mycobacterium tuberculosis* (Gautam et al., 2007). However, for the aqueous, hexane, acetone and methanol extracts, the MIC was greater than 200 µg/mL (considered not active) (Molina-Salinas et al., 2006).

Newton et al., 2000 provided a comprehensive review of the plant species used in folk medicine and tested them against *Mycobacterium tuberculosis*. Out of the 140 species described in that review, only *Bidens pilosa* was also tested in the present work. The leaf ethanol extract of *Bidens pilosa* exhibited activity against *Mycobacterium tuberculosis* at 100 µg/mL (Newton et al., 2000; Gautam et al., 2007). *Bidens pilosa* is also used in the Ayurvedic system of medicine to treat pulmonary diseases and leprosy (Gautam et al., 2007), and it is used in Ugandan traditional medicine for the treatment of TB and associated diseases (Tabuti et al., 2010) (Table 3).

Species of *Hymenaea* (*Hymenaea courbaril*, *Hymenaea intermedia* and *Hymenaea oblongifolia*) were commonly noted in a survey of the *quilombola* communities of Oriximiná, Pará, Brazil to treat cough, hoarseness, pneumonia and TB. However, the species *Hymenaea courbaril* was not active in the bioassay against *Mycobacterium tuberculosis* (Oliveira et al., 2011) (Table 3).

Mentha piperita is used in the Ayurvedic system of medicine to treat coughs and chest complaints (Newton et al., 2000; Gautam et al., 2007). However, the leaf methanol extract of *Mentha piperita* exhibited a MIC above 500 µg/mL against *Mycobacterium avium* and *Mycobacterium smegmatis* (Newton et al., 2000) (Table 3).

Vernonia polyanthes (“assa-peixe”) has been used in Brazilian traditional medicine to treat respiratory diseases (Oliveira, 2006). The antimycobacterial activity of a hydroalcoholic extract from the roots was evaluated against *Mycobacterium tuberculosis* strain H₃₇Rv and proved inactive (MIC higher than 4000 µg/mL (Oliveira, 2006).

Most species sold in open-air markets in the state of Rio de Janeiro, despite their indication and commercialisation to treat TB and associated symptoms, have not been assessed for antimycobacterial activity in any assays, which may indicate the potential for discovering new drugs in ethnobotanical studies of open-air

markets. Lall and Meyen (1999) affirmed that not much attention has been given to the laboratory evaluation and detection of antimycobacterial activity in South African medicinal plants. In the present work, we observe that plants commercialised in open-air markets in Brazil have not gotten much attention, despite the high demand for them by consumers.

3.4. Bioassay-guided fractionation of active hexane extracts from *Struthanthus marginatus* and *Struthanthus concinnus*

Based on the results of the ethnobotanical survey of plant sellers in this study (Table 2), *Struthanthus marginatus* and *Struthanthus concinnus* (Loranthaceae) were purchased to assess their antimycobacterial activity. We prepared extracts and assayed for activity against *Mycobacterium tuberculosis*. The results are displayed in Table 4. According to the literature, plant extracts with MIC values lower than 200 µg/mL are generally considered active (Tosun et al., 2004; Oliveira et al., 2011). In contrast, some authors only consider MIC results under 100 µg/mL to be positive (Borges-Argaez et al., 2007; Gautam et al., 2007). Still, others use a value of MIC ≤ 125 µg/mL (Molina-Salinas et al., 2007; Luo et al., 2011). Following this rationale, the activity of plant extracts can be classified as significant (MIC < 100 µg/mL), moderate (100 < MIC ≤ 625 µg/mL) or weak (MIC > 625 µg/mL) (Tekwu et al., 2012). The results in Table 4 show that the aqueous extracts prepared according to the informants' recipes were not active *in vitro* against either strain of *Mycobacterium tuberculosis*. However, ethanol extracts from both plants exhibited a moderate (100 µg/mL, *Struthanthus marginatus*) to good (25 µg/mL, *Struthanthus concinnus*) *in vitro* activity against *Mycobacterium tuberculosis* H₃₇Rv. We fractionated them by liquid–liquid partition to create new extracts that were also assayed. The hexane extracts from both plants were selected for bioassay-guided fractionation, which resulted in sterols, triterpenes and other terpenoids (Leitão et al., 2013). Some of the isolated or identified compounds have already been reported to be active against *Mycobacterium tuberculosis*: phytol, previously isolated from *Lucas volkensii*, exhibited very high activity against *Mycobacterium tuberculosis* (MIC: 2 µg/mL) (Cantrell et al., 2001); taraxasterol, isolated from *Chrysanthemum morifolium*, showed an MIC value of 64 µg/mL (Akihisa et al., 2005); and sitosterol (MIC 128 µg/mL) and stigmasterol (MIC 32 µg/mL), isolated from *Morinda citrifolia* (Okunade et al., 2004), and lupeol (MIC 64 µM), isolated from *Chuquiraga ulicina* (Cantrell

Table 4

Antimycobacterial activity of extracts and compounds isolated from hexane extracts of *Struthanthus marginatus* (SMH) and *Struthanthus concinnus* (SCH) against the H37Rv and ATCC-35338 strains of *Mycobacterium tuberculosis*.

Extracts, fractions and pure compounds	<i>Mycobacterium tuberculosis</i> strains	
	H ₃₇ Rv	35338
<i>Struthanthus marginatus</i>	MIC	MIC
Ethanol extract	100	200
Hexane extract (SMH)	100	100
Dichloromethane extract	100	200
Ethyl acetate extract	Resistant	Resistant
Butanol extract	Resistant	Resistant
Aqueous extract (prepared according to the informants recipes)	Resistant	Resistant
SMH-7 (a mixture of 6,10,14-trimethyl-2-pentadecanone; phytol and lupeol, along with two unidentified triterpenes)	200	25
SMH-9 (sitosterol in mixture with a minor quantity of stigmasterol)	Resistant	200
3- <i>O</i> - <i>n</i> -acyl-lup-20(29)-en-3 β ,7 β ,15 α -triol	200	100
<i>Struthanthus concinnus</i>	MIC	MIC
Ethanol extract	25	25
Hexane extract (SCH)	50	25
Dichloromethane extract	50	50
Ethyl acetate extract	Resistant	100
Butanol extract	Resistant	Resistant
Aqueous extract (prepared according to the informants recipes)	Resistant	Resistant
SCH-15 (phytol, taraxasterol, β -amyryn, α -amyrenone and 24-methylenecycloartanol)	Resistant	100
SCH-16 (containing taraxerol)	200	100
SCH-17 (containing obtusifoliol)	Resistant	Resistant
SCH-18 (6,10,14-trimethyl-2-pentadecanone, γ -sitosterol, plus unidentified compounds)	200	100
SCH-19 (6,10,14-trimethyl-2-pentadecanone plus unidentified compounds)	200	100
SCH-20 (6,10,14-trimethyl-2-pentadecanone plus unidentified compounds)	100	100
Taraxerol (isolated from SCH-16)	> 200	> 200
Obtusifoliol (isolated from SCH-17)	50	> 200
Positive control		
Rifampin	$\leq 0, 007$	> 2

MIC values in $\mu\text{g/mL}$; H₃₇Rv (sensitive to rifampicin) and strain ATCC-35338 (resistant to rifampicin). Resistant—extracts and fractions resistant at 200 $\mu\text{g/mL}$.

et al., 2001) have demonstrated activity. These compounds have been detected by GC–MS (Leitão et al., 2013) in fractions of the hexane extract of the *Struthanthus* in this study which, however, did not show relevant activity *in vitro*. Perhaps their dilution in those fractions did not allow them to reach their inhibitory concentrations (Table 4).

β -Amyryn and taraxerol, also detected in *Struthanthus concinnus* (Table 4), have been previously reported to be inactive in tests with the H₃₇Rv strain (Akihisa et al., 2005).

Some of the compounds isolated from the plants had never been assayed *in vitro* against *Mycobacterium tuberculosis*. The compound 3-*O*-*n*-acyl-lup-20(29)-en-3 β ,7 β ,15 α -triol (isolated from *Struthanthus marginatus*) had a MIC value of 200 $\mu\text{g/mL}$ for the H₃₇Rv strain, which was considered inactive, and for the strain ATCC-35338, the MIC was 100 $\mu\text{g/mL}$, which can be considered moderate. In contrast, obtusifoliol, isolated from *Struthanthus concinnus*, was sensitive to strain H₃₇Rv, with a MIC value of 50 $\mu\text{g/mL}$, which can be considered significant (Table 4). To the best of our knowledge, the antimycobacterial activity of *Struthanthus* species and related substances is described here for the first time (Asres et al., 2001; Gupta et al., 2010; Mohamad et al., 2011).

4. Conclusions

Ethnobotanical surveys in Rio de Janeiro open-air markets clearly demonstrate the importance of the knowledge of medicinal plant traders for drug research and development. Some of the plants indicated as remedies for lung problems and cough had been examined in the research literature and found to be active. A Salience Index was successfully applied to the lists of species used to treat tuberculosis and revealed two *Struthanthus* species (*Struthanthus marginatus* and *Struthanthus concinnus*, Loranaceae) as important

ethnospecies (“erva-de-passarinho”). Despite the fact that their popular preparation form (teas) was not active *in vitro* against *Mycobacterium tuberculosis* (MIC higher than 200 $\mu\text{g/mL}$), their less polar hexane extracts were active, and showed the presence of terpenoids with moderate to significant activity. Additionally, both *Struthanthus* species were always indicated in combination with another plant species to treat TB, which may indicate that some type of interaction or synergism could be present.

In summary, we can infer that popular knowledge was generally supported by the biological assays.

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