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Antimycobacterial activity of some Brazilian indigenous medicinal drinks

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ABSTRACT

Tuberculosis (TB) is a very serious problem worldwide and the increasing number of multiple drugs resistant TB cases makes the search for new anti-TB drugs an urgent need. Indigenous knowledge about the use of native plants to treat illnesses has contributed to the discovery of new medicines. In this study, the antimycobacterial activity of seven medicinal drinks was assessed: *Ananas sativus* (hydroalcoholic fruit extract), *Aristolochia triangularis* (aqueous and hydroalcoholic leaf, root and stem extracts), *Bromelia antiacantha* (hydroalcoholic fruit extract), *Stryphnodendron adstringens* (hydroalcoholic bark extract), *Tabebuia ovellanedae* (hydroalcoholic bark extract), *Vernonia polyanthes* (hydroalcoholic root extract), all used by the Vanuíre indigenous community in the treatment of respiratory diseases. The activity was evaluated by using a time-to-kill assay, in which *Mycobacterium tuberculosis* H37Rv was cultured on Löwenstein-Jensen medium, after thirty minutes, one, three, six, twelve and twenty-four hours contact of the bacteria with each drink. Within half to one hour contact, the hydroalcoholic drinks of *A. triangularis, S. adstringens, T. ovellanedae* and *V. polyanthes* reduced the bacterial growth by 2 orders of magnitude in CFU/mL, and all bacterial growth was absent after three hours contact. In contrast, no mycobactericidal effect was detected in the aqueous extract of *A. triangularis* or in the hydroalcoholic beverages of *A. sativus* and *B. antiacantha*, even after twenty-four hours contact.

Keywords: Tuberculosis; indigenous drinks; *Aristolochia triangularis; Stryphnodendron adstringens; Tabebuia ovellanedae; Vernonia polyanthes.*

INTRODUCTION

Mycobacterium tuberculosis is responsible for more human deaths than any other single microbial species. Although there are anti-tubercular agents that are reasonably effective in treating tuberculosis (TB), the misuse of these agents has led to an increasing prevalence of multiple-drug resistant strains (Vieira et al., 2007). Therefore, it is necessary to prioritize the research on new drugs with activity against *M. tuberculosis*. The relation between the risk of developing TB and social-economic factors is widely known (Portal da Saúde, 2007) and the problem of TB directly affects the low-income indigenous communities (Basta et al., 2004). However, during a period of ten years (1994 to 2004) of an epidemiological survey carried out among 208 inhabitants of the Vanuíre indigenous community established in Arco-Íris, São Paulo State, Brazil, only one case of tuberculosis was notified. The medicinal plants, in the form of crude extracts, infusions or beverages, are widely used by this community to treat common infections.

In this context, we decided to evaluate the antimycobacterial activity of beverages used by this community.

After analyzing the responses to a questionnaire presented to members of the Vanuíre indigenous community, six plants and seven beverages were selected: *Ananas sativus* (boiled fruit extract); *Vernonia polyanthes* (roots hydroalcoholic extract); *Stryphnodendron adstringens* (bark hydroalcoholic extract); *Tabebuia ovellanedae* (wood or bark hydroalcoholic extract), *Bromelia antiacantha* (boiled fruit extract), *Aristolochia triangularis* (leaf, root and stem hydroalcoholic and aqueous extract). These beverages are used by this community for the treatment of respiratory diseases. They were tested against *Mycobacterium tuberculosis* H37Rv.

MATERIAL AND METHODS

Ethical aspects

The work had the approval of the Brazilian National Foundation of Health (FUNASA), of the Indigenous National Foundation (FUNAI), Bauru, SP and of the Ethics Commission of the University of West São Paulo (UNOESTE), Presidente Prudente, SP, Brazil.

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Plant materials

Fresh plant material (at least 500g) from each species selected (Table 1) was harvested in the western region of São Paulo State, near Arco Íris, SP, Brazil. The exact location is indicated in Table 1. The plant specimens were collected with the aid of the local indigenous community and exsiccates were prepared, classified and deposited at the University of West São Paulo (UNOESTE), Presidente Prudente, SP. Collected plants were used to prepare the medicinal drinks according to the popular methods. The sugarcane juice spirit "cachaça" (with 30% alcohol), used to prepare the hydroalcoholic formulations, was acquired from the local community.

Microorganism

Mycobacterium tuberculosis H37Rv - ATCC 27294 was used to evaluate the antimycobacterial activity of the beverages. The bacterium was stored on Lowenstein Jensen (DIFCO) medium slants until use.

Antimycobacterial kinetic assay

Time-killing curves (in triplicate assay) were obtained with serial, decimal dilutions (10^{-1} to 10^{-4}) of M. tuberculosis prepared from 10^6 CFU/mL culture in Middlebrook 7H9 broth (BBL): 1mL of the 10^{-1} and 10^{-2} bacterial dilutions were mixed into 9mL of the beverages, giving final bacterial concentrations of 10^{-3} and 10^{-4}, respectively. The mixture was kept shaking for contact periods of half, three, six, twelve and twenty-four hours, after which 0.1 mL of each beverage sample was inoculated on Löwestein - Jensen (L-J) medium (DIFCO) slants and incubated at 35-37ºC for 21 days, under weekly observation. To monitor the viability of the bacteria, 0.1mL of the 10^{-3}, 10^{-4} and 10^{-5} dilutions were also cultured on the L-J medium and incubated in the same way. The influence of the alcohol present in the beverages was also evaluated by determining the antimycobacterial activity of the sugarcane spirit used. Isoniazid (Sigma) was used as a reference drug to assay bacterial sensitivity .

RESULTS

On the basis of the questionnaire presented to members of the Vanuíre indigenous community, six plant species and seven formulations used to treat pulmonary diseases by this community were selected (Table 1). During this survey it was noted that mainly the older people of the community made use of these formulations. When the herbs were not used directly to cure infections, they were used to help in other pulmonary treatments.

Antimycobacterial activity results are presented in Table 2. The beverages prepared with *A. triangularis* (aqueous), *A. sativus* and *B. antiacantha* did not show any antimycobacterial activity. The number of viable bacteria remained similar to the control even after 24h mycobacteria/ beverage contact. However, the hydroalcoholic beverages

Table 1 - Details of plant species and medicinal drinks.

Scientific name	Popular name	Collecting site	Part of plant	Extract
Ananas sativus Schultz	Abacaxizinho	Tupi Paulista/SP	Fruit	hydroalcoholic
Aristholochia triangularis Cham	Cipó mil homem	Paranapanema/SP	Leaves, Roots and Stem	aqueous
Aristholochia triangularis Cham	Cipó mil homem	Paranapanema/SP	Leaves, Roots and Stem	hydroalcoholic
Bromelia antiacantha Bertol	Gravatá do Mato	Anaurilândia/MS and Teodoro Sampaio/SP	Fruit	hydroalcoholic
Stryphnodondron adstringens Mart	Barbatimão	Paraguaçu Paulista/SP	Bark	hydroalcoholic
Tabebuia ovellanedae Lor. Ex Griseb	Ipê-roxo	P. Prudente/SP	Bark	hydroalcoholic
Vernonia polyanthes Less	Assa peixe	Paranapanema/SP	Root	hydroalcoholic

Table 2 - Results of mycobacterial activity of beverages in CFU/mL [a, b]

Beverages	**Extraction**	**Colony-forming Units (CFU)/mL**				
		***½* hour (log)**	***1* hour (log)**	***3* hour (log)**	***6* hour (log)**	***12* hour (log)**
A sativus	hydroalcoholic	4	4	3.9	4	4.1
A.triangularis	aqueous	4.2	4.1	4	4	4
A.triangularis	hydroalcoholic	2	absent	absent	absent	absent
B.antiacanta	hydroalcoholic	3.9	4	3.9	4.1	4.1
S.adstringns	*h*ydroalcoholic	3.1	2.5	absent	absent	absent
T.ovellanedae	hydroalcoholic	1.9	absent	absent	absent	absent
V. polyanthes	hydroalcoholic	2.9	2	absent	absent	absent
Cachaça	30% alcohol	3.5	3	2	1.2	0.8

[a] control: $\log_{10}$(CFU/mL)= 4.2

[b] contact time in hours

prepared from *A. triangularis*, *T. ovellanedae* and *V. polyanthes* showed promising activity against *M. tuberculosis*, even after discounting the poisonous effect of the alcohol against viable cells of *M. tuberculosis* (see 'cachaça' in Table 2). The hydroalcoholic beverages of *A. triangularis* and *T. ovellanedae* showed reductions of two logarithmic orders of magnitude in the number of viable bacteria after thirty minutes mycobacteria/beverage contact (reduction of 100-fold in viability) and absence of viable mycobacteria after one hour contact. For hydroalcoholic beverages of *S. adstringens* and , 100-fold reduction was obtained in 1hour and, after three hours contact, no mycobacterial growth at all was observed.

DISCUSSION

Plants have provided many medicinal drugs in the past and remain a potential source of therapeutic agents to this day (Phillipson, 2003). The use of plants with medicinal action has grown, despite all the advances made in medicine, even in developed countries, due to several factors such as the confidence of the populations that use them, their ease of acquisition, and their low cost (Récio et al., 1989). Public scepticism about the ability of allopathic medicines to be free from adverse effects, or to cure chronic conditions, have contributed to consumer demand for high quality herbal medicinal products (Phillipson, 2003). Hence, indigenous plants are used worldwide as medicines, particularly in the developing countries.

A growing number of chemical and pharmacological studies is proving the effectiveness and the existence of medicinal properties in a great variety of plants. Antimycobacterial activity has been found in several species of plants and some classes of natural compound such as the terpenoids (Cantrell et al., 2001) and physalines (Pietro et al., 2000; Okunade et al., 2004) were indicated as responsible for the biological activity against *M. tuberculosis*.

In this study, we selected six plant species and seven formulations used by the Vanuíre indigenous community to treat pulmonary diseases. This community, consisting of 208 inhabitants, is established in Arco-Íris district, São Paulo State, Brazil. The community is composed of people belonging to four indigenous nations (Krenak, Kerena, Kaiwa and Kaigang), which come from several Brazilian regions.

Antimycobacterial activity was confirmed in the hydroalcoholic beverages from *A. triangularis, S. adstringens, T. ovellanedae* and *V. polyanthes.*

There are several species of *Aristolochia* that exhibit similar properties, and they are also used as antiseptic, antitussive, expectorant, antiasthmatic medicines, and to treat lung inflammation (Lopes & Humpfer 1997). *A. triangularis*, collected in the Brazilian states of Rio Grande do Sul (114) and Paraná (Lopes & Humpfer 1997), showed a significant presence of kaurene diterpenes. In an extensive review of natural products with anti-mycobacterial activity, Coop (2003) registered the valuable antitubercular action of diterpenes. Other secondary metabolites, such as the tetrandine (Lopes & Humpfer 1997) isolated from species of *Aristolochia*, also proved to have a strong antibiotic effect against *M. tuberculosis*. The leaves and roots of *V. polyanthes* have been used in traditional medicine, as a diuretic, balsamic and antirheumatic (Lorenzi, 2000). The effect of the crude extract of *V. polyanthes* on blood pressure and renal excretion

was studied by Romanezi et al. (2003).

Owing to its high concentration of tannin, the bark of *S. adstringens* is used in home-made medicine against hemorrhage, diarrhea, hemorrhoids, wounds and conjunctivitis (Lorenzi, 2002).

The phytochemical analyses of *Tabebuia* species revealed the presence of naphthoquinones, mainly lapachol, that shows antitumoral activity and antimicrobial activity (Lorenzi, 2002). The antibacterial activity of *T. impetiginosa* against *Helicobacter pylori* was reported by Park et al. (2006). Nevertheless, we could find no research about the antimycobacterial activity of *Tabebuia ovellanedae*.

In spite of the wide use of the *V. polyanthes, T. ovellanedae, S. adstringens* and *A. triangularis* species by the Vanuíre community, the activity against *M. tuberculosis* of these plants, especially in the beverages, has still not been reported. Another relevant result emerging in this research is that, when the aqueous and hydroalcoholic beverages of *A. triangularis* were compared, it was observed that only the hydroalcoholic extract was active against *M. tuberculosis*, indicating the lesser polar character of the active constituents. This can be explained by the high hydrophobicity of the mycobacterial cell wall (Rando et al., 2002).

It is notable that, according to Basta et al. (2004), the incidence of tuberculosis among the Surui Indians (Rondônia) was as high as 2519 per 100.000 inhabitants. This reinforces the idea that tuberculosis is an infect-contagious and social disease and that the indigenous community is very susceptible to tuberculosis. However, the low incidence of tuberculosis (only one case) in the Vanuíre community during ten years surveillance may be explained by their use of beverages with antitubercular activity.

RESUMO

Atividade antimicobacteriana de algumas bebidas medicinais indígenas

A tuberculose é um problema mundial sério e vem aumentando significativamente com o surgimento de novos casos de multi-drogas resistência necessitando-se pesquisar novas drogas. Os conhecimentos indígenas sobre o uso de plantas nativas para a cura de certas doenças têm contribuído para a obtenção de novos medicamentos. No presente estudo, foi avaliada a atividade anti-micobacteriana de sete bebidas medicinais indígenas: *Ananas sativus* (extrato hidro-alcoólico de fruta), *Aristolochia triangularis* (extrato aquoso e hidro-alcoólico de folhas, raízes desidratadas), *Bromelia antiacantha* (extrato hidro-alcoólico de fruta), *Stryphnodendron adstringens* (extrato hidro-alcoólico de casca), *Tabebuia ovellanedae* (extrato hidro-alcoólico de casca), *Vernonia polyanthes* (extrato hidro-alcoólico de raiz) usados pela comunidade indígena Vanuíre situada em Arco-Íris, Estado de São Paulo no tratamento de afecções respiratórias. A atividade antituberculose foi avaliada pela exposição do *Mycobacterium tuberculosis* H37Rv as beberagens por trinta minutos, um, três, seis, doze e vinte e quatro horas. Nos tempos de exposição de trinta minutos a uma hora as bebidas hidro-alcoólicas de *A. triangularis, S. adstringens, T. ovellanedae* e *V. polyanthes*, reduziram em $2\log_{10}$ a multiplicação bacilar (determinada em UFC/mL). A ausência de crescimento bacteriano foi verificado a partir da terceira hora de exposição da bactéria a bebida. Por outro lado, nenhuma atividade micobactericida foi verificada nas soluções aquosas de *A. triangularis* e nas bebidas hidro-alcoólicas de *A. sativusand, B. antiacantha* mesmo após 24 horas de exposição.

Palavras-chave: Tuberculose; beberagens indígenas; *Aristolochia triangularis; Stryphnodendron adstringens; Tabebuia ovellanedae; Vernonia polyanthes.*

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