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Antibacterial activity of *Caesaria Sylvestris*, *Schinus Terebinthifolius* and *Tabebuia Avellanedae* - three native brazilian tree species

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Abstract

The aim of this work was to evaluate the antimicrobial activity of *Casearia sylvestris* (guacatonga), *Schinus therebentifolius* (Aroeira) and *Tabebuia avellanedae* (Ipe Roxo) extracts. Three extraction solvents were used (water, ether and alcohol 70%) with three different concentrations: 10%, 20% and 30% plants weight/volume. The inhibitory effect of these extracts on the growth of pathogenic bacteria in domestic animals was evaluated. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27253, the disk diffusion method in paper Müller Hinton was used. The filter paper discs was soaked with 10 microliters of herbal solution. After 24 hours the growth halos were measured. The inhibition halos larger than 10mm were considered. Controls were made with each solvent extraction. Inhibitory effect was obtained on *Staphylococcus aureus* with the following extractions: *Tabebuia* hydroalcoholic 10%, 20% and 30%, *Tabebuia* ether 10%, 20% and 30%, *Tabebuia* decoction 20% and 30%, 30% *Schinus* ether, *Schinus* water-alcohol 20% and 30% ether and *Casearia* 10%, 20% and 30 %. The effect was not observed for *Escherichia coli* and *Pseudomonas aeruginosa* inhibitory. The extracts obtained from *Tabebuia avellanedae* had satisfied inhibitory on the *Staphylococcus aureus* in vitro, followed by those obtained from *Schinus therebentifolius* and *Casearia sylvestris*.

Keywords: *Casearia sylvestris*, *Schinus terebinthifolius*, *Tabebuia avellanedae*, antimicrobial activity.

Atividade antibacteriana da *Caesaria Sylvestris*, *Schinus Terebinthifolius* and *Tabebuia Avellanedae* – três espécies nativas de árvores brasileiras

Resumo

O objetivo deste trabalho foi analisar a atividade antimicrobiana dos extratos obtidos da *Caesaria sylvestris* (Guaçatonga), *Schinus therebentifolius* (Aroeira) e *Tabebuia avellanedae* (Ipê Roxo). Foram utilizados três solventes para extração (água, éter e álcool 70%), com três concentrações diferentes: 10%, 20% e 30% massa/volume das plantas. Avaliou-se o efeito inibitório destes extratos sobre o crescimento das seguintes bactérias patogênicas para animais domésticos: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 e *Pseudomonas aeruginosa* ATCC 27253. Utilizou-se o método de difusão em disco de papel no meio Müller Hinton. Os discos em papel poroso foram embebidos com 10 microlitros de solução fitoterápica. Após 24 horas de crescimento os halos foram medidos. Considerou-se efeito inibitório os halos maiores que 10mm. Os controles foram feitos com o solvente de cada extração. Obteve-se efeito inibitório sobre *Staphylococcus aureus* com as seguintes extrações: Tabebuia hidroalcoólica 10%, 20% e 30%, Tabebuia éter 10%, 20% e 30%, Tabebuia decocto 20% e 30%, Schinus éter 30%, Schinus hidroalcoólica 20% e 30% e Casearia éter 10%, 20% e 30%. Para *Escherichia coli* e *Pseudomonas aeruginosa* não foi observado efeito inibitório. Os extratos obtidos a partir da *Tabebuia avellanedae* apresentaram bom efeito sobre o *Staphylococcus aureus* in vitro, seguidos dos obtidos a partir de *Schinus therebentifolius* e *Caesaria sylvestris*.

Palavras-chave: *Caesaria sylvestris*, *Schinus terebinthifolius*, *Tabebuia avellanedae*, atividade antimicrobiana.

Introduction

According to DUARTE (2006), the investigations on natural products with antimicrobial activity increased significantly in the last years, reflecting problems caused by the antibiotics' indiscriminate use. The World Health Organization (WHO, 1998) esteems that 65% to 80% of the population in developing countries depends on medicinal plants as the only form of access to basic health care. However, according to GONÇALVEZ et al. (2005), the great majority of the plants usually employed as popular phytotherapies did not have their therapeutic potentialities indeed proven.

Casearia sylvestris SW (Flacourtiaceae), known as “Guaçatonga” or lizard Herb, is widely distributed over Brazil. Several are the medical applications in the popular human medicine referred by POTT e POTT, (1994) and RODRIGUES e CARVALHO, (2001) as being antidiarrhetic, antipyretic, antirheumatic and snake bites as well as in scientific literature (SASSIOTO et al., 2004; ARANTES et el., 2005) as antibacterial in dentistry and for wound healing.

Schinus terebinthifolius RADD. (Anacardiaceae) known as Brazilian pepper or “Aroeira-mansa”, is from South America with introduction in South Africa and Mauricius’ island. The ethnopharmacology (POTT & POTT, 1994; RODRIGUEZ & CARVALHO, 2001) and recent researches (DEGÁSPARI, 2004; LIMA et el., 2004; DEGÁSPARI et al., 2005) attribute antidiarrhetic, astringent, anti-inflammatory, antimicrobial features, wound healing and purgative activities to this plant.

Tabebuia avellanedae (Bignoniaceae) known as “Ipê – Roxo” or lapacho-tree is from South America and frequently used for furniture and housebuilding in Brazil. African descendants in Brazil used the infusion for back pain (ALBUQUERQUE, 2001). The main active component seems to be a naphtoquinone known as lapachol, with anti-ulcerogenic, antimicrobial, anti-inflammatory, antimalaric, wound healing, tripanossomicide, anti-psoriasis, antiviral, anti-schistosomiasis, antitumoral and immunomodulatory effects (FONSECA et al., 2003; SOUZA et al., 2005; CORRÊA et al., 2006, LIPINSKI et al., 2008; MOON et al., 2007).

The present study was undertaken to investigate the in vitro antibacterial activity of aqueous, ethanol water (hydro alcoholic 70%) and ether extracts with three concentrations, 10%, 20% and 30% weight/ volume of the plants. Results of the antibacterial activity are being reported in order to subsidize further investigations with special interest on new products development for the veterinary medicine.

Materials and methods

Plant collection and preparation

Leaves and branches were obtained from *Caesaria sylvestris* (voucher number 14688) and stem bark from *Schinus terebinthifolius* (voucher number 14687) from six different places on the experimental farm of Pontifícia Universidade Católica do Paraná – PUCPR, where the species are common and *Tabebuia avellanedae* (voucher number 14686) from three different sawmills from the metropolitan area of Curitiba (southern Brazil). Exsicattes of these

materials were deposited in the herbarium at the State University of Ponta Grossa. The collected stem bark was cleaned from unwanted foreign materials, chopped up into small pieces and dried; the small branches and leaves were deposited under shadow in a period of one week. The material was then stored in paper bags at environment temperature in the laboratory.

Test microorganisms

The microbial organisms used for the study were Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, Gram negative bacteria: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27253, obtained from the Laboratory of Microbiology of the State University of Ponta Grossa (UEPG). The bacterial strains were grown in nutrient broth (Difco Laboratories©, Detroit, MI) at 37 degrees Celsius and maintained on nutrient agar slants at 4 degrees Celsius.

Plant extraction

The coarsely leaves and little branches of *Caesaria sylvestris*, were chopped up in small pieces. The bark of *Schinus terebinthifolius* and the sawdust of *Tabebuia avellanedae* were extracted using three solvents (water, ethanol 70 %, ether) with three concentrations, 10%, 20% and 30% weight/volume of the plants. About 100 grams of each sample were extracted with a particular solvent using the following method: After 10 minutes of the plant ebullition in deionized water. The solution was immersed in hydroalcoholic (ethanol 70 %) solution, during 7 days at 37 degrees Celsius. The immersion in ether was made during 24 hours at 37 degrees Celsius. The extracts were filtered and packaged into brown glass bottles. (ethanol and ether extracts). The same procedure was done to obtain the decoction.

Antibacterial sensitivity testing

The extracts were used against gram-positive and gram-negative bacteria by paper diffusion method (Shafi, 1975), impregnating the paper disc (6 mm diameter) with 10µl of each extract. The paper discs were placed over previously inoculated Müller-Hinton agar plates and incubated.

Results and discussion

Aqueous decoctions of *Tabebuia avellanedae*, *Schinus terebinthifolius* and *Caesaria sylvestris* are used in Brazilian traditional medicine for several purposes, mainly as antimicrobial, anti-inflammatory and for wound healing (ALBUQUERQUE 2001; RODRIGUES e CARVALHO, 2001; BUENO et al. 2005). The material preparation followed the traditional application, except ether extract. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are commonly found in human’s infected wounds (KEMPFER et al. 2010) and skin of animals (ZADOKS et al. 2002).

The results of antimicrobial activities against *Staphylococcus aureus* from the extracts by using paper diffusion method are summarized in Table 1. Against *Escherichia coli*, only the Ethanol 70 % extract of *Schinus terebinthifolius* obtained an inhibitory effect of 8 mm. All extracts were totally inactive against gram-negative bacteria *Pseudomonas aeruginosa*. Ethanolic and dichloromethane extracts made from Egyptian *Schinus terebinthifolius* leaves induced inhibition zones against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas. aeruginosa* (EL-MASSRY et al. 2009).

TABLE 1. The radius of inhibition zone for *Staphylococcus aureus* of extracts from leaves and branches, stem bark and sawdust in different concentrations.

	Inhibition zone (mm)								
	Ethanol 70 % extract			ether extract			decoction		
% w/v	10	20	30	10	20	30	10	20	30
<i>S. terebinthifolius</i>	9	10	10	-	9	10	-	8	9
<i>C. sylvestris</i>	-	-	-	10	11	11	-	-	-
<i>T. avellanedae</i>	15	15	15	10	12	13	-	11	12

- : Absence of effect

Although the ethanol 70 % and ether extracts from *Schinus terebinthifolius* were actived against *Staphylococcus aureus*, the decoction did not corroborate with the DEGÁSPARI et al. (2005) and GONÇALVEZ et al. (2005) findings, who observed the presence of antimicrobial activity only from ethanolic extract. This result may be attributed to the antimicrobial effect of the tannins and essential oils of *Schinus terebinthifolius* (LIMA et al., 2004; DEGÁSPARI et al., 2005). Substances like tannins and essential oils both are better extracted by alcoholic-aqueous solvents than only water as a solvent. *Schinus terebinthifolius* is rich in tannins (LIMA et al., 2004; DEGÁSPARI et al., 2005; SIMÕES et al., 2007), which

components contributed more for the antibacterial effect; it should be further investigated.

Caesaria sylvestris was activated only in ether extract, possibly due to the presence of flavonoids, (SHINES et al., 1999), which are better extracted with polar solvents (SIMÕES et al., 2007). Recently, flavonoids have been responsabilized by SANCHES et al. (2005) for the antibacterial activity in an another common south-american tree (*Psidium guajava*) widely distributed over tropical countries, with good results against Gram-positive bacteria and no inhibition of Gram-negative bacteria. Our study confirms the investigation of GONÇALVEZ et al. (2005) when he worked with *Caesaria sylvestris* and *Staphylococcus aureus*.

Tabebuia avellanedae was the most active against *Staphylococcus aureus* using all three solvents. The observed antimicrobial activity may be due to the presence of different naphthoquinones among others (RIFFEL et al., 2002; FONSECA et al., 2003; SOUZA et al., 2005; CORRÊA et al., 2006; PEREIRA et al., 2006). However, none of the solvents used in our experiment is considered ideal for extraction of the naphthoquinones in Bignoniaceae, even though small amounts could have been extracted with these solvents (FONSECA et al., 2003; SIMÕES et al., 2007). SILVA et al. (2002) found antitumoral, antifungal and antiviral activities for naphthoquinones, but did not test his antibacterial activity. Testing methanolic and dichloromethane extracts of the *Tabebuia avellanedae* bark, ANIBAL (2007), found no antibacterial activity. The poor activity of the ethanol 70 % extract of *Schinus terebinthifolius* only against *Escherichia coli* might be due to intrinsic variables of the strains or difficulty to transpose actively the most complex cellular walls.

This communication aims to contribute to further investigations development, which could be directed to the products and techniques development applicable to small farmer's reality in organic animal production systems in tropical countries, mainly South America. These applications, used in traditional human medicine are not used in veterinary medicine so far. The phytotherapeutic potential of south-american flora presents relevant potential for veterinary medicine. The preparation of lotions and soaps with antimicrobial activity as described by KARERU et al. (2011) would be also possible.

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