Vol.48, Special n. : pp. 237-241, June 2005
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Chemical and Biological Evaluation of Rejects from the Wood Industry

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ABSTRACT

*This study aimed chemical characterization and microbiological evaluation of extracts obtained from the waste of woods marketed in Paraná State: Peroba-Rosa (*Aspidosperma *sp.), Roxinho (*Peltogyne *sp.), Jatobá (*Hymenaea *sp.), Curupixá (*Micropholis *sp.), Itaúba (*Mezilaurus *sp.), Cedrilho (*Erisma *sp.) and Imbúia (*Licaria *sp.), whose botanical identifications were based on anatomical studies. The extracts were prepared with different solvents, analyzed by TLC and UV/VIS techniques, and tested against:* Proteus mirabilis *ATCC15290,* Staphylococcus aureus *ATCC25923,* Escherichia coli *ATCC25922,* Enterobacter aerogenes *ATCC13048,* Micrococcus luteus *ATCC9341,* Klebsiella pneumoniae *ATCC13883,* Pseudomonas aeroginosa *ATCC27853,* Staphylococcus aureus, Streptococcus mutans *and* Bacillus cereus *isolated from the clinic. The ethanol extract from Peroba-rosa containing alkaloids showed activity against* P. mirabilis. *Itaúba, Jatobá and Imbúia methanol extracts containing phenolics, and the Roxinho ethyl acetate extract containing terpenoids and phenolics were active against* K. pneumoniae, M. luteus, E. coli, S. aureus *and* P. mirabilis. P. aeroginosa, S. mutans *and* E. aerogenes *were resistant to the extracts.*

Key words: Tropical woods, microbiological evaluation, chemical profile of woods

INTRODUCTION

The native wood exploration in Brazil is frequently predatory, and the explored areas have been transformed into pasture or simply abandoned. Currently, Paraná State is a major wood exporter in the country with USS 500,000.00 in the year of 2002 (AIMEX, 2004). This business is mainly based on *Eucalyptus* and *Pinus* species, but part of such export and the demand of the regional industry is met by the exploration of natural forests, especially of the Amazon Forest (Agência Estado, 2003).

The mechanical processing of these woods generates approximately 14% of lumber from the total volume of the logs. The residues are partially used to generate energy or they are displaced at inadequate areas, generating serious environmental problems (Mayan et al., 2003). The search for useful applications for these rejects of this industry is still a hard task to carry through. The secondary metabolites found in the lumbers can add value to such crude materials or it can be transformed into novel by-products of these residues, but it is necessary to study the specific chemical composition of each lumber.

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Many woods of high commercial value come from plant species with few or none chemical/biological study about their secondary metabolites. In a recent bibliographical search (ISI WEB OF KNOWLEDGE, 2003), only a few old chemical studies about Peroba-rosa (*Aspidosperma polyneuron*, alkaloids), Jatobá (*Hymenaea courbaril*, flavonoids and terpenoids), Imbúia-do-Norte (*Licaria* sp., neolignoids), Roxinho (*Peltogyne* sp., peltogynoids) and Itaúba (*Mezilaurus itauba*, neolignoids and essential oil) were found. The majority of these studies mentions the chemical composition of other parts of the tree, not of the wood itself. The woods of Cedrilho (*Erisma* sp.) and Curupixá (*Micropholis* sp.) were the most explored Amazonian species in the last years (AIMEX, 2004), but not even one scientific study on the secondary metabolites of these two botanical genera could be found.

A troublesome factor for the accomplishment of studies on rejects of the wood industry is that the logs arrive there only with a commercial identification or a popular name. The popular denomination of the Brazilian native trees is changeable from region to region. Sometimes different common names represent the same species, and same name can represent different species (Camargos et al., 2001). In these cases, the botanical identification of the genus can be made by anatomical analysis of the wood and by verifying characteristics such as color, flavor or smell (USDA, 2004). The intensification of scientific research on the wood of native species would lead to a more realistic valuation of their environmental role by those who explore them commercially. The chemical study of the secondary metabolites of woods and their microbiological activities may lead to a comprehensive understanding of their defenses against plagues, providing the scientific basis to the development of new protective products for white woods, for example.

MATERIALS AND METHODS

Wood Samples

Wood samples of seven species were supplied by a wood industry of the city of Ponta Grossa - PR, which are commercially identified as Peroba-rosa, Cedrilho, Imbúia-do-Norte, Itaúba, Roxinho, Jatobá and Curupixá. The botanical identification of the samples was made by macroscopical analysis of their color and anatomical characteristics, following the identification key of Mainieri (1983). For the microscopical analysis, permanent blades with safra-blau coloration were prepared by histologic cuts in the three plans (transversal, longitudinal tangential and longitudinal radial). The identifications were confirmed by comparison with samples of the Embrapa Florestas collection and with descriptions contained in specialized literature (Mainieri and Chimelo, 1989; Metcalfe and Chalk, 1965).

Preparation of the crude extracts

Lumber of each wood sample (100 g) of were dried at 40 ^{o}C during 3 days. After that the samples were extracted exhaustively in Soxhlet apparatus with dichloromethane and later with methanol, and then the solvents were evaporated under vacuum. Roxinho was also extracted with ethyl acetate and methanol. Peroba-rosa was extracted only with ethanol. Itaúba's lumber was extracted with a mixture of 80% chloroform and 20% methanol, and then with pure methanol.

TLC and UV/VIS Analyses

In the thin layer chromatography (TLC) analyses of the extracts, 5 μL of sample solution made up from 10 mg of crude extract for 1 mL of solvent were applied on silica gel HF-254 plates eluted with different systems of solvents. After the development, the chromatograms were observed under UV light of 254 and 360 nm, and then sprayed with specific reagents for terpenoids (anisaldehyde/H_2SO_4), phenolics ($FeCl_3$) and alkaloids (Dragendorff). Qualitative UV/VIS spectra of the extracts were measured in methanol. Each measure of spectrum was repeated after adding two drops of a NaOH solution (5%), to confirm the presence of phenolic compounds by the occurrence of batochromic shifts. The spectrum in acid medium was also obtained with the extract of Peroba-Rosa for a comparison with literature.

Microbiological activity of the extracts

The method used for the microbiological evaluation was the diffusion in solid-medium by plate-cavity (Cleeland and Squires, 1991). The bacterial suspensions were standardized by the 0.5 MacFarland tube and inoculated by "pour-plate" on Tryptic Soy Agar medium (Oplustil et al., 2000). In cavities of 6 mm located in the middle of

the culture medium, the dissolved extracts were placed using a concentration of 1 mg/cavity. After incubation at 37 ºC for 24 h, the diameter of the halo of inhibition around the cavity was measured. For the microbiological tests, the extracts were dissolved in DMSO. The tested bacteria used in this study were: *S. aureus* ATCC 25923, *P. mirabilis* ATCC 15290, *E. coli* ATCC 25922, *E. aerogenes* ATCC 13048, *B. cereus* and *S. mutans* isolated from the clinic. The extracts from Roxinho wood obtained with ethyl acetate, Jatobá's and Itaúba's methanolic extract, Peroba's ethanolic extract and Itaúba's extract obtained with $CHCl_3$/MeOH 20% were tested against *M. luteus* ATCC 9314, *K. pneumoniae* ATCC 13883, *P. aeroginosa* ATCC 27853 and *S. aureus* isolated from the clinic. The experiments have been carried out three times using equivalent volumes of DMSO as negative control.

RESULTS AND DISCUSSION

The wood samples were identified as botanical genera: Peroba-rosa (*Aspidosperma* sp., Apocynaceae), Cedrilho (*Erisma* sp., Vochysiaceae), Imbúia-do-Norte (*Licaria* sp., Lauraceae), Itaúba (*Mezilaurus* sp., Lauraceae), Roxinho (*Peltogyne* sp., Caesalpinaceae), Jatobá (*Hymenaea* sp., Caesalpinaceae) and Curupixá (*Micropholis* sp., Sapotaceae). The yield of the extracts that have been tested against the bacteria are shown in Table 1.

In Table 2 the results of the TLC analyses and detection of compound classes are summarized. In some cases, these results supported the identification of the botanical genus carried through by the anatomical studies of the woods.

Strong alkaloid presence in the crude ethanol extract of Peroba-Rosa was observed, that was a natural class of compounds occurring in all species of the *Aspidosperma* genus. UV spectra of this extract showed great similarity with the literature values for polyneuridine, the main indole alkaloid isolated from *Aspidosperma polyneuron* (Antonaccio et al., 1962). These results strongly suggested that wood sample of Peroba-Rosa came from *A. polyneuron*. This tree is found in the Atlantic Forest in Bahia, Espírito Santo, São Paulo and Paraná States; it also occurs in Mato Grosso do Sul, Minas Gerais, Goiás, Mato Grosso and Rondônia States, in the semideciduous latifoliated forest (Lorenzi, 1992).

The observed phenolic compounds in the Itaúba extracts probably corresponded to lignoids already isolated from the *Mezilaurus* genus (Yaez et al., 1986); bisbenzylisoquinoline alkaloids were found in this botanical genus (Hisses et al., 1983).

Table 1 - Yield of the extracts.

WOOD	yield (%)
Itaúba, MeOH	5.80
Itaúba, $CHCl_3$/MeOH 20%	2.86
Peroba Rosa, MeOH	7.80
Imbuía-do-Norte, MeOH	6.38
Cedrilho, MeOH	7.58
Curupixá, MeOH	8.94
Jatobá, MeOH	17.5
Roxinho, AcOEt	1.68
Roxinho, MeOH	10.1

Table 2 - Compound classes found in the extracts.

WOOD	phenols	terpenes	alkaloids
Itaúba	+	+	+ (weak)
Peroba Rosa	+	-	+ (strong)
Imbúia	+	+	-
Cedrilho	-	+	-
Curupixá	+	+	-
Jatobá	+	+	-
Roxinho	+	+	-

Lignoids are also characteristic of species from the *Licaria* genus, here represented by the Imbúia-do-Norte (Braz Filho et al., 1981). The volatile monoterpenoid anethole, isolated from *Licaria* species, is known for its antimicrobial properties (Himejima and Kubo, 1992). Some species of the *Peltogyne* genus, the one to that Roxinho belongs, have their wood endowed with violet coloration due to phenolic compounds known as peltogynoids, which are flavonoids (Almeida et al., 1974). The trees of the *Hymenaea* genus (Jatobá) are characterized by its resins with high ratio of diterpene acids, which are strong antimicrobials (Abdel-Kader et al., 2002); flavonoids of varied structures are also common in *Hymenaea* species (Pettit et al., 2003).

The results of the microbiological tests indicated that ethanol extract of Peroba-rosa had strong inhibitory effect against *Proteus mirabilis*. Methanol extracts of Itaúba, Jatobá and Imbúia-do-Norte, as well as the extracts of Roxinho obtained with ethyl acetate and Itaúba with $CHCl_3$/MeOH 20%, all inhibited the growth of the Gram positive and Gram negative bacteria to some extent. The Curupixá's, Roxinho's and Cedrilho's methanol extracts showed weak or no antibacterial activity.

The main relative results of the microbiological tests with the extracts are summarized in Table 3; the values, given in mm, are the diameters of inhibition halos provoked by the presence of each extract in the culture medium. *E. aerogenes*, *P. aeroginosa* and *S. mutans* were resistant to all the extracts tested.

Table 3 - Main results of microbiological tests; averages of the observed inhibition halos (mm).

Extracts	Ec	Kp	Ml	Pm	Sa	ob
Itaúba (MeOH)	0	11	10	0	11	--
Jatobá	14	13	13	14	14	0
Imbúia-do-Norte	11	--	--	12	11	--
Roxinho	0	--	--	0	10	--
Roxinho (AcOEt)	9	10	9.5	10	9	0
Cedrilho	0	--	--	0	0	--
Peroba Rosa	0	9	9	21	9	0
Curupixá	0	--	--	0	0	--

■= not tested; **Ec** = *E. coli*; **Kp** = *K. pneumoniae*; **Ml** = *M. luteus*; **Pm** = *P. mirabilis*; **Sa** = *S. aureus*; **ob** = other bacteria tested: *P. aeroginosa*, *S. mutans* and *E. aerogenes*

ACKNOWLEDGEMENTS

We are grateful to the Programa PIBIC UEPG/CNPq for an IC grant (Daniel Granato) and to CNPq for a Research grant (Domingos Sávio Nunes, 308591/2003-8). We also thank to Dr. Paulo C. Botosso at Embrapa Florestas, Colombo - PR, for his participation in the botanical identification.

RESUMO

Este estudo visa a caracterização química e a avaliação da atividade antimicrobiana de extratos obtidos a partir de rejeitos resultantes do beneficiamento de madeiras nobres comercializadas no Paraná: Peroba-Rosa (*Aspidosperma* sp.), Roxinho (*Peltogyne* sp.), Jatobá (*Hymenaea* sp.), Curupixá (*Micropholis* sp.), Itaúba (*Mezilaurus* sp.), Cedrilho (*Erisma* sp.) e Imbúia-do-Norte (*Licaria* sp.), cujas identificações botânicas basearam-se em estudos anatômicos. Os extratos foram preparados com diversos solventes, analisados por CCD e espectrometria UV/VIS, testando-se contra: *Proteus mirabilis* ATCC15290, *Escherichia coli* ATCC25922, *Enterobacter aerogenes* ATCC13048, *Staphylococcus aureus* ATCC25923, *Micrococcus luteus* ATCC9341, *Klebsiella pneumoniae* ATCC13883, *Pseudomonas aeroginosa* ATCC27853, e *Staphylococcus aureus*, *Streptococcus mutans* e *Bacillus cereus* isolados da clínica. O extrato etanólico de Peroba-Rosa, contendo alcalóides, apresentou atividade contra *P. mirabilis*. Os extratos metanólicos do Jatobá, Itaúba e Imbúia, contendo fenóis, e o extrato de Roxinho obtido com acetato de etila contendo fenóis e terpenóides, foram ativos contra *K. pneumoniae*, *M. luteus*, *E.*

coli, *S. aureus* e *P. mirabilis*. Nenhum dos extratos foi ativo contra *P. aeroginosa*, *S. mutans* e *E. aerogenes*.

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Received: September 29, 2004;
Revised: February 25, 2005;
Accepted: March 25, 2005.