CONTROL OF BIODETERIORATION USING A FRACTION ISOLATED FROM LEAVES OF RICINUS COMMUNIS LINN.

de la Paz J[1]., Larionova M[2]., Maceira MA[2]., Borrego SF[1]., Echevarría E[1].

[1]Laboratorio de Conservación Preventiva. Archivo Nacional de la República de Cuba. Compostela 906 Esquina San Isidro. Habana Vieja. C.P. 10100. Ciudad de La Habana. Cuba. Teléfono: (537) 8629436 E-mail: delapazjn@yahoo.es
[2]Departamento de Investigaciones Médico Militar. Instituto Superior de Medicina Militar "Dr. Luís Díaz Soto". Ave. Monumental y Carretera del Asilo. C.P. 11700. Teléfono: (537) 954251. E-mail: ismmds@infomed.sld.cu

Summary

In archives, especially in those that are located in tropical clime, biodeterioration causes increasing damages every year to founds, which leads occasionally to the irreversible lost of documents heritage that is treasured in it. The different controlling methods are often chemicals that have limitations due their costs and to the negative impact they can cause on the environment and on the substrate that receives it. In virtue of this situation, search of alternatives as the rational use of natural products that come from plants has been done. That is why the purpose of this work was to evaluate the effect of an isolated fraction from *Ricinus communis* Linn on isolated bacterial from indoor environments of archives as well as in the control of rodents. The evaluation fraction (FRC2A) was obtained using ethyl acetate extraction from an aqueous extract of leaves of *Ricinus communis*. The raticide effect was determinate by the Medial Lethal Dose (LD_{50}) oral administration in 70 hybrids mouse $B_6D_2F_1$ while the bactericide effects was evaluated by growth inhibiting method by diffusion of the product on agar holes, using strain of *Enterobacter agglomerans*, *Bacillus polimixa* and *Streptomyces* sp. for this essay. The calculated LD_{50} was 388.08 mg/Kg and the concentrations that showed the highest antimicrobial activity were 50 and 100 mg/mL. The obtained effects could be related with the presence of ricinine and different flavonoids in the evaluated fraction.

Key words: *Ricinus communis* Linn, Antimicrobial activity, Biodeterioration, Natural products, Plants extracts

Introduction

Medicinal plants are an important therapeutic aid for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (1). Their antimicrobial activity has been known for millennia since in China, India, then later in Egypt and Greece different parts of medicinal plants have been used to cure specific ailments. Also, raticide and insecticides properties have been detected in plant so many years ago (2).

Usually, in the biodeterioration control has been used chemical products (biocides) but they are toxic and frequently their degradation is difficult, being persistent in the natural environment and causing contamination of areas distant from the site of treatment. However, the use of plant in the biodeterioration control is recent (3).

The use of natural compounds, such as plant extracts that will be biodegraded more easily and be more environmentally acceptable is advantageous. Many of these compounds are phenols, terpenoids, essential oils, alkaloids, polypeptides, flavonoids and other substances (4).

The aim of this paper was to study the effect of one fraction of *Ricinus communis* on the toxicity in mice and antimicrobial activity against different bacteria isolated from indoor air of repositories of documents in National Archive of Cuba.

Materials and Methods

Isolation of the FRC2A from leaves Ricinus communis Linn

The leaves of *Ricinus communis* Linn were collected in Havana City (Cuba). After it were dried and milled, and the powder obtained was extracted with hot water. The extract was evaporated in vacuum until a ¼ of volume. It was extracted with EtOAc. After evaporated of the EtOAc, the residue was dissolved in hot ethanol and left to cool. The crystals thus obtained were recrystallized three times, dried at 40 ºC. The ethanol solution was evaporated in vacuum and obtained FRC2A. The identification of crystals was determinate by IR spectroscopic method. Also, it was realized a chromatographic in TLC system (Kieselgel 60 F254): EtOAc: MetOH: H_2O (100:17:13); detection: $ALCL_3$ 5 % in MetOH, UV 366 nm to FRC2A. In it were used rutine and recinine as patron.

Determination of LD_{50}

The acute toxicity of fraction isolated (FRC2A) from the leaves of Ricinus communis Linn was determinated in male mice hybrids $B_6D_2F_1$ (18 – 20 g). The animals were fasted 8 hours prior to the experiment. Animals were administered with single dose of fraction and observed for its mortality 48 hours study period toxicity (short-term). The LD_{50} was calculated using Litchfield and Wilcoxon Methods.

Determination of the antimicrobial activity

Enterobacter agglomerans (Gram negative bacteria), *Bacillus polimixa* and *Streptomyces* sp. (both Gram positive bacteria) isolated from the indoor air of repositories of National Archive of Cuba were used. These strains were maintained in Nutrient Agar slant for 24 h. The suspensions of the bacterial strains correspond to 1 x 10^6 CFU/ml of the Mc Farland scale.

Antimicrobial activity of the fraction FRC2A was determined by the diffusion method with holes (5). Petri dishes with 15 ml of sterile Nutrient Agar were seeded with the appropriate bacterial suspension. Then in the holes (5 mm) 10 μL of the extract of different concentration were added. One additional hole - with sterile water- was included in the test as controls. After incubation for 24 h at 28 ºC, the plates were observed for zones of growth inhibition, and the diameter of these zones was measured in millimeters. Additionally, and for comparative purposes, thymol (2%) was included in the test. Each test was performed in triplicate.

Results

The identification of ricinine in the crystals was deduced from spectroscopic data in comparison with literature report. The IR spectrum showed an intense band at 2222 cm^{-1} attributed to the nitrile group and the band relative to the carbonyl group was at 1659 cm^{-1}. In the TLC system were showed more that 7 elements predominate between they ricinine and rutine.

The LD_{50} was estimated at 388.08 mg/Kg. for oral administration of the fraction (linear regression from a log-logit plot; r = 0.95). No effect was observed after vehicle administration. Before death the animals became exophtalmic, presented muscular tremors, and sometimes walked dragging the hid paws. In all cases of sudden death, after the animals stopped breathing their hearts continued beating for several minutes. The effects were significant at doses of 500 mg/Kg. The animals died in the first 2 hours after receive the drug (Table 1).

Table 1. Estimation of the LD_{50} of FRC2A

GROUP	DOSE mg/Kg	UNITS	TOTAL	VALUE OBSERVED	VALUE WAITED
1	250	0	10	0.12	0.41
2	300	1	10	10	6.11
3	350	2	10	20	26.76
4	400	4	10	40	57.21
5	450	9	10	90	81.29
6	500	10	10	97.88	93.59

It was possible to obtain a high antimicrobial activity when the concentration of the fraction was the highest (100 mg/mL). The activity was decaying when the concentration of the extract diminished (Table 2).

Table 2. Average inhibition halo diameter (mm) at different concentrations of fraction FRC2A.

Concentration	*Enterobacter agglomerans*	*Bacillus polimixa*	*Streptomyces* sp.
100 mg/mL	10	5	8
50 mg/mL	10	3	6
25 mg/mL	5	0	1
12.5 mg/mL	0	0	0

Discussion

The phytochemical analysis of the fraction FRC2A showed the presence the flavonoids rutine and the alkaloid ricinine. The data obtained is consistent with literature reports. Farah (6) suggested that ricinine might inhibit the mitochondrial respiratory chain and Pérez (7) reported that flavonoids may responsible for the antimicrobial activity.

The antimicrobial activity of *Ricinus communis* had been determined previously (8-10). Some of these authors have reported that this plant has antifungal activity. However, an insignificant activity was determined by Upasani et al. (9).

In this work a moderate activity was observed. However, the activity for *Enterobacter agglomerans* was higher than other bacteria. Possibly because *E. agglomerans* is a Gram negative bacteria and it seems to be the most sensitive of all. Likewise, *Bacillus polimixa* was the most resistant in all the bacteria. This result was not surprising, in general, this bacterial genera is the most resistant than Gram positive bacteria (11) (Lennette, 1985. In the case of *Bacillus polimixa* it is worth mentioning that it is a spore-forming species.

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