DIFFERENTIAL SENSITIVITY OF *BACILLUS* sp. ISOLATED FROM ARCHIVE MATERIALS TO PLANT EXTRACTS

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Summary

Books, photographs, maps and other paper-based documents are vulnerable to damage caused not only by high temperature, excess humidity or light, but also by activity of microbial contaminants. Libraries and archives have traditionally relied on the use of chemicals for both routine prevention and observed infestation. However, these chemicals often fail to prevent infestation, and their subsequent use cannot repair the damage. Apart from this, use of chemicals is currently less due to growing awareness of their dangerous health effects on personnel using them, the potential for damage to collections treasured in these

institutions, and environmental pollution. Modern methods such as use of plant extracts have shown promising results for the prevention and treatment of microbial contaminants in archival materials. The goal of this work is to study the antimicrobial activity of extracts from *Cichorium intybus* L., *Arctium lappa* L, *Centaurea cyanus* L , *Plantago major* L., *Medicago sativa L.*, *Allium sativum* L., *Eucalyptus citriodora* Hook, *Pinus caribaea* Mor and *Piper auritum* Kunth, against *Bacillus* sp., a microorganism isolated from photographs deposited in the Historical Archive of Museo of La Plata, Buenos Aires, Argentina. Diameter of inhibition halos varied according to the plant extract used.

Keywords: Biodeterioration, *Bacillus* sp, Antimicrobial activity, Plant extracts, Photographic paper, Inhibition halo.

Introduction

The alterations produced by microorganisms on items deposited in archives include modifications of the physical, chemical and mechanical properties of the materials, in addition to aesthetic changes. The intensity of deterioration depends on the composition of the support material, the environmental conditions and the associated microorganisms.

The genus *Bacillus* is frequent in archives and libraries; it can attack cellulose, parchment and glues, causing deterioration of the documents. This is more frequent in the case of antique papers, where these microorganisms subsist basically on glue and other non-fibrous components. In combination with phycomycetes, these bacteria can cause significant damage, since they decompose the substances produced by the cellulolytic action of the fungi. Likewise, they produce metabolites such as amylase, cellulase and lactic acid, which cause alterations indicated by acidity, purplish stains and brittleness of paper. Under high relative humidity-high temperature conditions, these microorganisms are capable of breaking down paper in 24 hours (1,2).

The preservation of the documents deposited in libraries and archives is extremely important because they safeguard the graphic memory of nations for the future generations.
Some of the most widespread treatments for the control of microorganisms growth include the use of chemical biocides (3, 4, 5). However, the effect of extracts obtained from plants on the microorganisms that damage the materials held in archives, libraries and museums, has been little explored. Such substances are used successfully as biocides in the medical, food and pharmaceutical industry fields, among others (6, 7, 8). Plant extracts have different modes of action: regulation of intermediate metabolism, activation or blocking of enzymatic reactions, direct effects on enzyme synthesis, or alteration of membrane structures (9). The goal of this work is to study the antimicrobial activity of extracts from plants that occur in Argentina and Cuba, against *Bacillus* sp.

Experimental

Plant material and preparation of extracts

The following plant species were used in this study: *Cichorium intybus* L. (chicory), *Arctium lappa* L. (great burdock) y *Centaurea cyanus* L. (cornflower, bacheror's button) (Asteraceae), *Medicago sativa* L. (alfalfa) (Fabaceae) and *Plantago major* L. (common plantain) (Plantaginaceae), collected from the wild in La Plata, Buenos Aires, Argentina in June 2006, and *Eucalyptus citriodora* Hook (lemon-scented gum tree) (Myrtaceae); *Pinus caribaea* Morelet (Caribbean pine) (Pinaceae); *Allium sativum* L. (garlic) (Liliaceae) and *Piper auritum* Kunth (Veracruz pepper, Mexican pepperleaf) (Piperacea), collected in La Habana, Cuba, between November 2005 and March 2006.
These plants were selected for their known antimicrobial activity and on the basis of their high content of secondary metabolites such as coumarins, flavonoids, organic acids and tannins, among others (8, 10, 11, 12).
The plants were first rinsed in water and then oven-dried at 60º C for 24 h, stored at room temperature and later ground using a mechanical grinder. The extracts and oils were obtained from

the aerial parts of plants. For extracts, 70% and 99% ethanol was used as solvent, preservative or both, and a 10-day maceration period (13). The extracts were first filtered using double-layered gauze to eliminate large particles and then using Whatman N°1 filter paper,Whatman, England. Lastly, the extracts were sterilized by filtering through Millipore 0.22 µm membrane filters. The essential oil from *Piper auritum* Hook was obtained by hydrodistillation from dry leaves; 5% and 10 % test solutions were prepared using 70% ethanol.

Sampling, isolation and identification of microorganisms

Samples were taken from the surface of photographic paper deposited in the Historical Archive of Museo of La Plata, Argentina, using sterile cotton swabs, and placed in sterile plastic containers for subsequent laboratory analyses. Then, these samples were homogenized in 10 mL of sterile saline physiological solution, and cultured in Petri dishes according to plate count or colony count techniques, and using different culture media such as nutritive agar, plate count agar and CPS medium for the growth of heterotrophic mesophilic bacteria and proteolitic bacteria (14, 15). Incubation time was 48 - 72 hrs.

The colonies isolated from the different culture media were classified using Gram staining. Mossel agar was used for isolation of aerobic sporulated microorganisms.

Of the microorganisms identified, *Bacillus* sp. was selected for further analyses because it was present in all the samples.

The environmental parameters for the Archive at the time of sampling were as follows: Temperature: 23.8 °C, Relative Humidity: 59%, Lighting: indirect artificial light (protected area), Air conditioning: natural ventilation; Personnel access: limited.

"In vitro" essay for antimicrobial activity

The effectiveness values of the differents concentrations of the used extracts were estimated by MIC (Minimun Inhibiting Concentration).

The antimicrobial activity of each extract was determined by hole-plate diffusion methods (16). The inoculum used for this

technique corresponded to tube 3 of the Mc Farland scale ($1x10^6$ CFU/mL). 10 μL of the extract were added in holes of 5-mm of diameter. Controls were set using equivalent volumes of sterile water, 70% alcohol and 40 mg/mL gentamicin sulphate (IMEFA, Cuba). The holes were equidistant (6 peripheral holes/plate). After 24-hour incubation at 28 °C, the plates were examined for inhibition growth. Halo diameter was measured in mm, not including hole diameter. All essays were performed in triplicate.

Results and Discussion

The Table 1 shows the diameter of resulting inhibition halos (in mm). The assessment of inhibition zones through the hole-plate diffusion methods showed that the extracts were effective against the tested strain, with the exception of *Allium sativum* L. and *Plantago mayor* L (inhibition halos less than 6 mm) (17).

Previous research has shown that the antimicrobial activity of plant extracts is due to different secondary metabolites present in the plants, including: essential oils, triterpenoids, flavonoids, phenols, alkaloids, coumarins, tannins and steroids ([8, 9, 16, 19, 20, 21 22, 23, 24, 25), some of which occur in the extracts studied here and would be responsible for their antimicrobial effect against *Bacillus* sp.

The absence of antimicrobial effects in the case of *Allium sativum* could be due to the fact that the activity of allicin and ajoen, two metabolites with strong antimicrobial effects, is limited by their structural instability. Furthermore, sporulated Gram-positive microorganisms such as *Bacillus* have always shown greater resistance to antimicrobials (22, 25). In any case, the presence of these active principles was not quantified in this work; furthermore, these are not the only two active principles with putative antimicrobial found in galenic preparations made from garlic.

Concerning the antimicrobial activity of *Eucalyptus* sp., it has been attributed to the presence of tannins, terpens and eucalyptol in its leaves.

Table 1: Mean and standard deviation (in mm) of inhibition halos for the extracts tested against *Bacillus* sp.

Plant species	Alcohol content %	Inhibition halo (mm)	Activity
Allium sativum L	70	0	negative
Arctium lappa L.	70	X: 12.3 DS: 0.6	positive
	99	X: 15.0 DS: 0.9	positive
Centaurea cyanus L.	70	X: 7.3 DS: 0.5	moderate
	99	X: 7.6 DS: 0.7	moderate
Cichorium intybus L.	70	X:6.0 DS:0.2	moderate
	99	X:6.0 DS:0.3	moderate
Eucalyptus citriodora Hook	70	X:13.0 DS:0.3	positive
Medicago sativa L.	*70*	X:6.0 DS:0.3	moderate
	99	X:6.0 DS:0.2	moderate
Plantago major L.	70	0	negative
	99	0	negative
Pinus caribaea Mor	70	X:9.0 DS:0.3	moderate
Piper auritum Kunth 5%		X:8.0 DS:0.3	moderate
Piper auritum Kunth 10%		X:10.0 DS:0.3	positive
Distilled water		0	negative
Ethanol 70 %		0	negative
Gentamicin sulphate 40 mg/mL		X: 16.8 DS:0.4	positive

Negative: less than 6 mm Moderate: between 6 and 9 mm
Positive: more than 9 mm

Similar results were reported (26) when evaluating an hydro-alcoholic extract of *Arctium lappa* obtained by maceration at low temperatures, against *Staphylococcus aureus*, *Streptococcus pyogenes* type A and *Streptococcus pyogenes* type B. This effect was attributed to the arctiopicrin (sesquiterpenic lactone) present in the aerial parts of this plant.

With respect to the effects of plantain, literature sources report antifungal activity of a cream made from its leaves, in a concentration of 20.7 g of solids per gram of hydrophilic ointment, effective against *Candida albicans* and, to a lesser degree, against *Trichophytum rubrum.* Such concentration is much higher than the one used in this study. Lack of effects against other germs has also been reported (27).
Generally, a biocidal effect was observed in the case of most of the extracts assayed, which would support a promissory use of these substances for the control of microbial deterioration. However, further studies are necessary to evaluate the innocuousness of these plant extracts for the permanence and durability of paper. In addition, the plant origin of many of the colorants, agglutinants, adhesives and consolidants that have been used to make sheets and documents from the origins of paper manufacture to the present, must be taken into account (2)

Conclusion

The results obtained confirm the antimicrobial activity of these plant extracts and would support a more extensive use of these substances to control microbiological deterioration in archive materials.

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