

NATURAL PRODUCTS ISOLATED FROM PLANTS USED IN
BIODETERIORATION CONTROL

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Summary

Biodeterioration designates the physical and chemical alterations resulting from the biological activity that takes place on a surface. The communities of microorganisms associated to biodeterioration grow as biofilms that adhere to the substrates. The treatment used for control of the problems related to biodeterioration and biofouling in materials of patrimonial value are based on avoiding or diminishing the formation of biofilms. The biocides used for these purposes are chemical agents that are mostly toxic; many are difficult to degrade and persistent in the environment. An increasing interest exists for the regulation of the indiscriminate use of toxic chemical compounds. Natural products with biocidal activity, obtained from plants, represent an alternative and useful source in the control of biodeterioration, without negative environmental impacts. The aim of this work was to evaluate the antimicrobial activity of different aqueous, acetic, and ethanol extracts of *Cichorium intybus* L., *Arctium lappa* L., *Centaurea cyanus* L. (Asteraceae), *Allium sativum* L. (Liliaceae), *Pinus caribea* Mor. (Pinaceae), *Eucalyptus citriodora* Hook. (Myrtaceae) and *Piper auritum* Kunth (Piperaceae) against different microorganisms associated with biodeterioration. The results of the extracts obtained are described and analyzed, and the potential value of these natural products in the control and prevention of biodeterioration is discussed.

Key words: Antimicrobial activity, Biodeterioration, Natural products, Plant extracts.

Introduction

In indoor and outdoor environments a great number of particles of different origin can be found, they constitute the atmospheric aerosol and they have different forms and size. These particles can be deposited on the surfaces which can be different kind of materials or alive and dead organisms being formed this way the biofilms (1). These biofilms accumulate in engineered systems such as heat exchangers, pipelines, processing equipments, and storage tanks, and induce technical problems including reduction in heat transference, biocorrosion and associated environmental damages and product contamination (2). In the case of biodeterioration of different structural materials of patrimonial heritage, including archives, photographic paper, wood, leather, etc., in which biofilms also occur, the economic cultural losses are incalculable.

The prevention of biodeterioration should be mainly focused on avoiding or minimizing the development of microorganisms and the consequent biofilms formation. Chemical treatments applied to biodeterioration control involve the use of biocides and other products such as different paints. Unfortunately, biocides are inherently toxic and are frequently difficult to degrade, being persistent in the natural environment or able to accumulate in a variety of matrixes, and often causing contamination of areas distant from the site of treatment (3). Thus biocides may have a very negative impact on the environmental if they are applied without a proper environmental risk assessment.

One innovative attempt is the use of naturally-produced compounds, such as plant extracts that will be biodegraded more easily and be more environmentally acceptable (4-8). Many of these compounds are phenols and polyphenols, terpenoids and essential oils, alkaloids, lectins, and mixtures of polypeptides and other substances (9).

The aim of this paper was to study the antimicrobial activity of aqueous, acetic and ethanol extracts of *Cichorium intybus*, *Arctium lappa*, *Centaurea cyanus* (Asteraceae), *Allium sativum* (Liliaceae), *Pinus caribea* (Pinaceae), *Eucalyptus citriodora* (Mirtaceae) and *Piper auritum* (Piperaceae) against different microorganisms isolated from different materials of patrimonial and cultural interest.

Methods

Plant materials

To perform this study the following plants were collected: “Chicory” (*Cichorium intybus* L.), “Bardana” (*Arctium lappa* L.) and “Centaura” (*Centaurea cyanus* L.) all of them Asteraceae. All the plants were collected on June 2006 from an uncultivated field at the locality of La Plata, Buenos Aires, Argentina, on a day with temperature of 16 °C. The field had light soil, with good development of humus, humid (rain had fallen 2-3 days before) with anthropic modification. Healthy-looking aerial parts and roots were collected.

These plants was selected for their bacteriostatic activity (Chicory), given by their content in coumarins (cichoriin), flavonoids (kaemperol), organic acids (chlorogenic and isochlorogenic acids), for their antimicrobial and bactericide activity (Bardana), given by their content in polyacetilenic compounds, sesquiterpens (arctiopocrin), organic acids (acetic and ascorbic acids), and for their antimicrobial activity (Centaura) given by their content in flavons and flavonoids, coumarins (cichoriin) and tannins, among other components (10, 11, 12).

Taxonomic determination was performed and voucher specimens (Chicory AM-101, Bardana AM-102, Centaura AM-103) were deposited in LEBA Scientific Collections. In other hand *Allium sativum*, *Pinus caribea*, *Eucalyptus citriodora* and *Piper auritum* were collected in Havana City (Cuba) between November 2005 and March 2006 and its leaves used for obtain the extracts.

Samples procedures

From biofilms: The biofilms were removed from the surface of different materials, such as paper and silk maps and photographs deposited in the National Historical Archive, Havana, Cuba, using a cotton swab. Samples were immediately placed into sterile plastic vessels and taken to the laboratory for later analyses. Physical parameters obtained in these areas were: Map Repository: T: 24 °C, Relative Humidity (RH): 52 %, Insolation: Indirect Light, Air conditioned. Photograph Repository: T: 16 °C, RH: 50 % Insolation: indirect light (protected area), Air conditioned. Concurrence of personnel: limited.

Airborne microbiological sampling: The air samples were collected from two repositories using an impactation slot biocollector (slot II, 30 L.min⁻¹). The biocollector was placed at 1.5 m of height, following a diagonal sampling design. Petri dishes with Nutrient Agar were used for bacteria isolation. Physical parameters obtained in these areas were: Repository 11: T: 26°C, Relative Humidity (RH): 60 %, Insolation: Indirect Light, Natural ventilation. Repository 13: T: 27 °C, RH: 67 % Insolation: indirect light, Natural ventilation (protected area). Concurrence of personnel: limited.

Isolation and identification of microorganisms

The samples obtained from biofilms were aseptically suspended in 10 ml sterile saline solution; later, a 1 ml-aliquot was cultured on different culture media in Petri dishes.

Nutrient Agar, PCA and CPS media, were used to grow heterotrophic mesophilic bacteria (13). For physiological groups the following media were used: i) lipolytic, lipase activity; (tributirine agar), ii) amylytic, amylase activity; (starch agar), and iii) proteolytic, protease activity; (gelatine agar Frazier) (14). Postgate B was used for sulphate-reducing bacteria, DRCM for sulphite-reducing bacteria and YGC for fungi and yeasts (13). The incubation time was 48-72 h for bacteria and one week for fungi, both incubating at 28 °C. In the case of bacteria, the colonies isolated from different culture media were Gram stained. Gram negative bacteria were isolated using EMB agar and Cetrinide agar, and spore forming bacteria were isolated using Mosel agar. *Pseudomonas fluorescens*, two different species of *Pseudomonas*, *Bacillus cereus*, *Bacillus polimixa*, *Enterobacter agglomerans* and *Streptomyces* sp. were identified. For all samples, observations under light microscope at 40 X showed bacteria and scarce filamentous cells.

Microbial cultures and standard agar disc diffusion method (SDA)

Pseudomonas fluorescens, *Pseudomonas* sp., *Bacillus cereus*, *Bacillus polimixa*, *Enterobacter agglomerans* and *Streptomyces* sp. were maintained in nutrient agar slant for 24 h. The suspensions of the bacterial strains correspond to 1×10^6 CFU/ml of the Mc Farland scale.

Antimicrobial activity of the aqueous and ethanol extracts of different plants was determined by the disc diffusion method (15). Petri dishes with 15 ml of sterile nutrient agar were seeded with the appropriate bacterial suspension. Sterile 6 mm diameter Whatman filter paper discs were impregnated with 100 % concentrations of "Cichory", "Centaura" and "Bardana" ethanol extracts and placed on the seeded plate. Two additional sterile blank discs - one soaked with sterile water and one with ethanol - were included in the test as controls. Likewise, in holes of 5mm of diameter 10 μ L of Allium (tincture, 29.9 mg/mL), Pinus caribea (tincture, 22.2 mg/mL), Eucaliptus (fluid extract, 45.8 mg/mL) and Piper (essential oil, 10%) were added. Two additional holes -one with sterile water and other with ethanol 70%- were included as controls. After incubation for 24 h at 28 °C, the plates were observed for zones of growth inhibition, and the diameter of these zones was measured in millimetres. Additionally, and for comparative purposes, commercially available coumarin aqueous solution and coumarin ethanol solutions (concentration 10^{-3} M) and thymol (2%) were included in the test. Each test was performed in triplicate.

Preparation of plant extracts

Plant materials were dried in an oven stove at 60° C during 24 h and stored at room temperature until further processing. Dried plant material (aerial parts and roots) was finely ground with a crushing machine.

Dried plants were extracted with acetone, ethanol, ethanol 70% and sterilized water. The ethanol and acetonic extracts (Merck 99 % of purity) were obtained by weighing 10 g of ground and stabilized plant material. For maceration, the ethanol and acetonic extracts were placed in 100 ml of the respective solvent during 7 or 10 days at room temperature, keeping the extracts in the dark room and stirring them manually 3 times a day. The aqueous extract was prepared by adding 100 ml to 10 g of ground and stabilized plant material, and the mixture was left standing for 20 min. The aqueous extract was filtered and freeze-dried. The acetonic and ethanol extracts were filtered and the filtrate was evaporated using a rotary evaporator until it acquired syrupy consistency, and the residue were then dissolved in the respective solvent to obtain the final concentration. The essential oil of *Piper auritum* was obtained by hydrodistillation.

The extracts were filtered by double gauze to eliminate the heaviest material and then filtered using Whatman filter paper N° 1 (Whatman, England). The extracts were sterilized by filtration through 0.45 µm Millipore membrane filter.

Results

The diameters of the inhibition zones obtained with the extracts are presented in Table I and II

Table I. Average inhibition halo diameter (mm) for the different drugs tested.

Extracts / Microorganisms obtained from biofilms	<i>Cichorium intybus</i> L	<i>Arctium lappa</i> L	<i>Centaurea cyanus</i> L
<i>Bacillus cereus</i>	0	0	0
<i>Pseudomonas sp</i>	16	16	14
<i>Pseudomonas fluorescens</i>	0	0	0
<i>Pseudomonas sp</i>	12	10	16

Table II. Average inhibition halo diameter (mm) for the different drugs tested.

Extracts / Microorganisms obtained from air	<i>Allium sativum</i>	<i>Pinus caribea</i>	<i>Eucalyptus citriodora</i>	<i>Piper auritum</i>
<i>Enterobacter agglomerans</i>	5	5	5	5
<i>Bacillus polimixa</i>	0	2	2	0
<i>Streptomyces sp.</i>	6	8	3	8

Discussion

The antimicrobial activity from bacteria isolated from biofilms was present only in the ethanol extracts. Traditionally, plant extracts are prepared with water (for example infusions, decoctions, etc.) so it seems unlikely that the traditional medicinal products extract the compounds responsible for activity in the ethanol extract (17).

Aqueous and organic extracts from the same plants show different activities. There is no common rule for this, but in most cases, the organic extracts show equal or greater activity than the aqueous extracts (5). In this case, the aqueous extract did not show any antibacterial activity. *Cichorium intybus*, *Arctium lappa* and *Centaurea cyanus* showed moderate activity against two different species of *Pseudomonas* and showed no antimicrobial activity against *Bacillus cereus* (Table I). Likewise, another preparation of different plant shows a moderate activity against so much Gram positive bacteria as Gram negative bacteria obtained from air, showing *Bacillus polimixa* the biggest resistance (Table II). This result was not surprising, as, in general, these bacteria are more resistant than Gram positive bacteria (18, 19). In the case of *Bacillus cereus* and *Bacillus polimixa* it is worth mentioning that it is a spore-forming species. These last results (Table II) are related with the metabolites presents (14).

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