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# Biological properties of the Chilean native moss *Sphagnum magellanicum*

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**ABSTRACT**

An ethanol extract prepared from the gametophyte Chilean native moss *Sphagnum magellanicum* was dried out, weighed and dissolved in distilled water. This extract was then assayed for its antibacterial activity against the G(-) bacteria *Azotobacter vinelandii*, *Erwinia carotovora* subsp. *carotovora*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi, Vibrio cholerae*, and the G(+) bacteria *Staphylococcus aureus* subsp. *aureus,* and *Streptococcus* type β. The growth of the cultures of *E. carotovora* subsp. *carotovora*, and *V. cholerae* was inhibited at a concentration of 581μg/ml of extract, while the cultures of *E. coli*, *S. typhi* and *Streptococcus* type β were inhibited at a concentration of 1.16 μg/mL of extract. The concentration of phenolic compounds was 4.294 mg/mL; the presence of vanillic, chlorogenic, syringic, caffeic, gallic, 3-4 hydrozybenzoic, p-coumaric and salicylic acids was identified using RP- High Pressure Liquid Chromatography.

**Key terms:** *Sphagnum magellanicum*, phenolic compounds, antibacterial activity, MIC.

## 1. INTRODUCTION

The moss species, *Sphagnum magellanicum* Brid. (Sphagnaceae), in its natural form, is found in Chile from parallels 37º 34' 58.88"S to 44º 02' 11.90"S South Latitude. It grows in extensive areas of an edaphic formation called "ñadi" and in wetlands.

This species has the great ability to absorb and retain water up to twenty times its dry weight, a characteristic that has been utilized for industrial and agricultural purposes (Van Breemen, 1995; Villaroel et al., 2002). It is considered a source of dietary fiber and has other properties as well: regulation of intestinal functioning by reduction of glucose and may also attributes that prevent colon cancer (Atalah et al., 1999). The antimicrobial properties of the ethanol extract have been studied in various species of *Sphagnum* (Basile et al., 1999) and in the Indian native moss *Sphagnum junghuhnianum* (Singh et al., 2006). In relation to these studies, the objective of the present investigation was to study the effects of ethanol extracts of Chilean native moss *S. magellanicum* on the *in vitro* growth of the G(-) bacteria: *Azotobacter vinelandii*, *Erwinia carotovora* subsp. *carotovora*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi, Vibrio cholerae*, and the G(+) bacteria: *Staphylococcus aureus* subsp. *aureus* and *Streptococcus* type β. Furthermore, the phenolic compounds present in the ethanol extract, which may have antioxidant capacity, were also studied.

## 2. METHODS

### *2.1. Vegetative Material*

The moss was taken from a wetland in Quillaipe (41°31'43.89"S South Latitude,

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72°44'43.63"W West Longitude) in the province of Llanquihue in the Los Lagos Region of Chile.

*2.2. Preparation of ethanol extract of S. magellanicum resuspended in distilled water*

Six point six grams of *S. magellanicum* were processed, dried and crushed using a Wathman Nº 2 paper filter bag. The continuous extraction was performed in the Soxhlet apparatus using 250 mL of ethanol 80%v/v for 3 hours. The extract was distilled at reduced pressure in a rotating evaporator at 45 ºC (Del Valle y Valdevenito, 1999). The extract was suspended in 2mL of ethanol, passing it through a chromatographic column of Amberlite XAD-2 (200mm in height, 20mm in diameter). The graduated cylinder was filled with 200mL of distilled water, discarding the overflowing fraction. Afterwards, the graduated cylinder was filled with 200mL of 100% ethanol (J.T. Baker®, HPLC grade). This was done in order to obtain the phenolic compounds that are found adhering to the chromatographic column. This mixture was dried out with a rotating evaporator. The dry extract was suspended in 3mL of triple distilled water (Montenegro and Salas, 2006; Montenegro and Salas, 2007). The extract was sterilized by filtering with a syringe through filter paper with a pore size of 0.2 μm (Orange® Scientific, Gyro disc CA-PC). The final concentration of the extract was 6.2mg/mL.

The absence of microorganisms in the extract was verified by seeding 100μL of extract in Petri dishes prepared with Soy Agar Trypticase and then incubated for 48 hours at 37º C in an incubator. There was no detection of bacterial colony growth; and the assay was done three times.

*2.3. S. magellanicum extract effects on the in vitro growth of G (-) and G (+) bacteria.*

The study was made with the bacterial culture collection of the Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. The stock G (-) bacteria studied were: *Azotobacter vinelandii*, *Erwinia carotovora* subsp. *carotovora*, *Enterobacter aerogenes* ATCC 13048 (American Type Culture Collection), *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* STH 2370, and *Vibrio cholerae* ISP (isolated from a collection from the Instituto de Salud Pública de Chile). The G (+) bacteria studied were: *Staphylococcus aureus* subsp. *aureus* ATCC 25923 and *Streptococcus* type β. The bacterial cultures were kept isolated in Petri dishes with Soy Agar Trypticase (BBLTM Trypticase™ Soy Broth and Agar) in an incubator in darkness at 37ºC for 48 hours. The aim was to allow bacterial multiplication until the moment of use.

*2.4. Determination of the minimal concentration of the extract for bacterial growth inhibition (MIC)*

The determination of MIC is defined as the minimal extract concentration required inhibiting bacterial development. This was determined in micro plaques with 96 trays with a plane base of 8 rows (A-H) and 12 columns (Orange Scientific®). The micro dilution method was utilized (Pontino et al., 2006). In every well 150 μL of Trypticase Soy Broth were placed (BBLTM Trypticase™ Soy Broth), and then in the first column another 150 μL of extract was added and then by half dilution was completed within the same column. Once the dilution was completed, 50 μL of bacterial suspension of 1.5 x $10^6$ ufc/mL in distilled water and equivalent to 75000 colony forming units per well, measured as 0.005 McFarland turbidity units (Biomeriux, Chile), were placed in every well except the control. The final extract dilution is expressed in μg/mL of solution (Table 1). Some rows of the plaques were used for controls (without bacteria inoculums). The plaques were incubated at 37º C for 24 hours, and the bacterial growth within each well was evaluated according to turbidity.

*2.5. Determination of antioxidant properties of S. magellanicum extract* To determine the antioxidant properties of *S. magellanicum* extract, the reduction method of free radical

DDPH (1,1-Diphenyl-2-2-picrylhydrazyl) was utilized. 950 μL of DPPH solution in ethanol [(measured at 517 nm in a spectrometer, ESPECTR BID-1, Agilent 8453) with an absorbance of 0.6 (DO)] and50 μL of *S. magellanicum* ethanol extract were placed in quartz cubets. This measurement was repeated three times. The samples were homogenized and the absorbance of the mixture was measured immediately. The results were expressed in mmol of DDPH reduced/mL of extract (Oszmianski et al., 2007; Liu et al., 2007). The phenolic compound concentration of *S. magellanicum* was evaluated according to the following methodology. An aliquot of 40 μL of extract was placed inside an Eppendorf tube (1.5 mL capacity), 100 μL of reactive Folin-Ciocalteu and 560 μL of distilled water was added to the tube and allowed to incubate for 15 minutes at room temperature. This procedure was repeated three times. After the incubation, 300 μL of a calcium carbonate solution (7%) was added to stop the reaction. Finally, the absorbance of the mixture was measured using a wavelength of 660 nm (Liu et al., 2007).

The characterization of the phenolic compounds was done using a reversed-phase high performance liquid chromatographic (RP-HPLC, Agilent 1000 series, G1316A COLCOM, serial # DE43646850) under the following conditions: 20μL of ethanol extract were injected for 20 minutes at 25° C, pressure at 95 bar and a flow of 1mL/min. A second characterization of the phenolic compounds was done using a reversed-phase high performance liquid chromatographic (RP-HPLC, LaChrom serie 7000 Merck-Hitachi (Darmstadt, Germany), a Symmetry C18 Waters (Ireland), 4,6x250mm, 5mM, Pre-column: Symmetry C18 3.9 x 20 mm,5 μm,) under the following conditions: 20μL of ethanol extract were injected for 31 minutes at 40ºC, using phase mixture A:2% formic acid mixture B: 3 2% formic acid: Methanol, 1:1 v/v mixture C: Methanol UV-wave Detection at 280nm, 310nm and 360nm. Compounds were quantified using peak area calculation, and partially identified using correlations between retention times.

## 3. RESULTS

*3.1. Antibacterial activity of S. magellanicum ethanol extract:* The *S. magellanicum* extract inhibited the *in vitro* growth of *V. cholerae* and *E. carotovora* subp. *carotovora, E. coli, S. typhi* and *Streptococcus* type β. The extract did not

TABLE 1

Antibacterial activity of the *S. magellanicum* ethanol extract, expressed in minimal extract concentration required to inhibit *in vitro* growth (MIC, μg/mL), on distinct isolated bacteria

| Bacteria | Bacterial Inhibition | MIC of *Sphagnum* extract per bacteria species μg/ml |
|---|---|---|
| *Erwinia carotovora* subsp. *carotovora* | + | 581.25 |
| *Vibrio cholerae* | + | 581.25 |
| *Escherichia coli* | + | 1162.5 |
| *Salmonella typhi* | + | 1162.5 |
| *Streptococcus* type β | + | 1162.5 |
| *Azotobacter vinelandii* | _ | _ |
| *Enterobacter aerogenes* | _ | _ |
| *Pseudomonas aeruginosa* | _ | _ |
| *Staphylococcus aureus* subsp. *aureus* | _ | _ |

+: Bacterial inhibition, -: No bacterial inhibition.

inhibit the *in vitro* growth the bacteria *A. vinelandii, E. aerogenes, P. aeruginosa and S. aureus* subsp. *aureus* (Table 1).

*3.2. Antioxidant capacity, concentration and characterization of phenolic compounds:* The antioxidant capacity of the *S. magellanicum* extract was 0.179 mmol of DPPH reduced/ml extract, which is equal to 841 μmol TROLOX/100g of *S. magellanicum* with a total phenolic concentration of 4.22 mg of total phenols/ml of extract. Through high-pressure liquid chromatography (HPLC), the presence of vanillic acid, salicylic acid and other compounds was determined (Table 2).

TABLE 2

Phenolic compounds present in the ethanol extract of the native moss *Sphagnum magellanicum*, detected by RP-HPLC

| Name | Total/area[mAU*s] | Area |
|---|---|---|
| Dyhidroxybenzoic | 330.90744 | 0.4665 |
| Chlorogenic acid | 395.94772 | 0.5582 |
| Sculetin | 95.11459 | 0.1341 |
| Caffeic acid | 133.92627 | 0.1888 |
| Syringic acid | 203.30341 | 0.2866 |
| Scopoletin | 76.30556 | 0.1076 |
| Vanillic acid | 567.51276 | 0.8001 |
| Salicylic acid | 88.05146 | 0.1241 |
| Total | | 2.6659 |

## 4. DISCUSSION

The *S. magellanicum* extract had an important antibacterial effect against *E. carotovora* subsp. *carotovora, V. cholerae, E. coli, S. typhi* and *Streptococcus type* β, nevertheless, the extract concentrations required were significantly higher (500 to 1100μg/ml) than those reported for the ethanol extracts of eight dry species of native moss from India: *Sphagnum junghuhnianum*, *Barbula javanica*, *Barbula arcuata*, *Brachythecium populeum*, *Brachythecium rutabulum*, *Minium marginatum* and *Entodon* cf. *rubicundus*. The MIC in these ethanol extracts varied between 0.2 and 6.5 μg/mL (Singh et al., 2006). This could indicate that the antibacterial agents of *S. magellanicum* are found in inferior quantities than those of the Indian moss, or could be due to different extraction systems. The promising results of this work correspond to the first study conducted on this native Chilean Bryophyte species and is a good start for continuing research involving other Bryophyte species with bacteriostatic properties. Furthermore, a moss extract whose habitat is the non-contaminated south of Chile makes it very attractive for use in the control of human and plant pathogens and as an organic food product.

The characterization by RP-HPLC (High Pressure Liquid Chromatography) determined the presence of phenolic compounds: Vanillic acid, syringic acid, chlorogenic acid, gallic acid, 3-4 hydroxybenzoic acid, caffeic acid, p-coumaric acid and salicylic acid. These components could be responsible for the antibacterial activity. Although the mechanisms involved are either not reported or not known in the majority of cases, this idea is based on the information found in the numerous publications reporting antibacterial activity in plant extracts and other organisms which also contain these phenolic compounds (Brent,2003; Cushnie and Lamb 1999). For example, vanillic acid has antibiotic properties against yeast that causes decomposition of foods such as *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii*; salicylic acid, the precursor to aspirin, inhibits the growth of *Helicobacter pylori* and *Staphylococcus aureus* (Kaellman, 1994).

The antioxidant capacity of the *S. magellanicum* extract obtained was 0.18 mmol of DDPH reduced/mL of extract, equivalent in ORAC (Oxygen radical absorbance capacity assay) units to 841 μmol Trolox/100g units. This value of activity is inferior to the antioxidant capacity found in vegetative species for human consumption, such as garlic (*Allium sativum*) with 5.3-5μmol TROLOX/100g and broccoli *(Brassica oleracea* cv. *italica*) with 3.53 μmol TROLOX/100 g. The

antioxidant capacity for *S. magellanicum* is similar to the capacity reported in cabbage cultivar "Corazón de buey" (*Brassica oleracea* cv. *capitata capitata*), 856 μmol of equivalents TROLOX/100g, and is superior to vegetables like pumpkin (*Cucurbita pepo*), 396 μmol TROLOX/100g (Ninfali et al.,2005).

Examples of other plant extracts that have shown lower levels of antioxidant capacity, such as extracts from the root of the vegetative species, *Filipendula vulgaris* (Rosaceae), native to Poland, have an activity of 720 μmol of TROLOX/100g equivilants (dry material). *Waldsteinia geoides* has an activity of 440 μmol of Trolox/100g equivilants (dry material) (Osmianski et al., 2007).

Some studies have described the possibility of using *Sphagnum magellanicum*, as a source of fiber for foods, as a functional food, that is a food consumed not only for its nutritional characteristics, but also to achieve specific functions (Roberfroid, 2000). This is because of its total dietary fiber content, which reaches 77%, a level much higher than found in other known fiber sources, such as rice shells, oats, barley and lupines (Villaroel et al., 2002). This study showed that Chilean native moss *S. magellanicum* has antibacterial and antioxidant properties.

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