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1 **Antibacterial activity of a sulfated galactan extracted from the marine alga**
2 ***Chaetomorpha aerea* against *Staphylococcus aureus*.**

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8 **Abstract**

9 The *in vitro* antimicrobial activity of the marine green algae *Chaetomorpha aerea* was
10 investigated against gram-positive bacteria, gram-negative bacteria, and a fungus. The water-
11 soluble extract of algae was composed of a sulfated (6.3%) galactan with a molecular weight
12 of 1.160×10^6 Da and a global composition close to commercial polysaccharides as dextran
13 sulfate or fucoidan. The polysaccharide was composed of 18% arabinose, 24% glucose, 58%
14 galactose. The re-suspended extracts (methanol, water) exhibited selective antibacterial
15 activities against three gram-positive bacteria including *Staphylococcus aureus* (ATCC
16 25923). Minimum inhibitory concentration and minimum bactericidal concentration tests
17 showed that the sulfated galactan could be a bactericidal agent for this strain ($40 \text{mg} \cdot \text{mL}^{-1}$).
18 Results of the present study confirmed the potential use of the green algae *Chaetomorpha*
19 *aerea* as a source of antibacterial compounds or active known molecules.

20 **Keywords:** Seaweed, *Chaetomorpha aerea*, sulfated galactan, antibacterial activity,
21 *Staphylococcus aureus*

22

23 INTRODUCTION

24 Seaweeds are used by coastal populations for thousands of years owing to their high
25 nutritional values [1, 2]. However, the industrialization of seaweeds does not necessarily need
26 their consumption. Medical and pharmaceutical industries are also interested since marine
27 plants are rich in active molecules [3, 4]. Indeed, the therapeutic potentials of certain
28 substances are extremely promising, especially as antimicrobial and antiviral factors [5, 6].
29 Besides, the use of fucoidans allows fighting against the formation and growth of malignant
30 tumors [7, 8]. Numerous studies have investigated the biological activities of algae extracts
31 [9]. Different seaweeds, e.g. *Ulva fasciata* or *Enteromorpha compressa*, have presented
32 antimicrobial activities against *Staphylococcus aureus* or *Pseudomonas aeruginosa*, two
33 bacteria commonly found in many human infections [10]. Nevertheless, microorganisms are
34 able to adapt their metabolism for resisting to the action of antimicrobial drugs [11, 12]. This
35 problem is one of the main reasons which require further research of new antimicrobial
36 compounds, including molecules from marine algae [13]. *Chaetomorpha aerea* is a green
37 filamentous alga which develops in many marine mediums as oyster ponds. Numerous species
38 of *Chaetomorpha* have often been studied since these organisms behave like opportunistic
39 macrophytes, causing evident ecological changes in the ecosystem contaminated. In French
40 oyster ponds (Marennes-Oléron, France), the excessive proliferation of these seaweeds
41 prevent the proper development of the phytoplanktonic portion that oysters need to grow up.
42 More generally, different studies have attempted to positively exploit these algae. It was
43 reported that *Chaetomorpha linum* was able to chelate heavy metals (copper and zinc) in
44 aqueous solutions [14]. A heparin-like polysaccharide has been highlighted in the seaweed
45 *Chaetomorpha antennina* [15]. Finally, the biological properties of sulfated arabinogalactans
46 [16, 17] extracted from green algae like *Chaetomorpha* were investigated. In this way, an

47 interesting polysaccharide composed of arabinose (57%), galactose (38.5%), rhamnose (3.8%)
48 and sulfates (11.9%) was purified from *Chaetomorpha linum* [18].

49 In the present study, we have developed a method to extract extracellular polysaccharides
50 from *Chaetomorpha aerea*. These polysaccharides were studied and biochemically
51 characterized to determine their composition and their partial structure. We have also
52 investigated the potential antimicrobial activities of these different extracts against
53 microorganisms, i.e. *Staphylococcus aureus* (ATCC 25923), *Salmonella enteritidis* (ATCC
54 13076), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (CIP 103214),
55 *Bacillus subtilis* (CIP 5262), *Micrococcus luteus* (ATCC 4698) and *Candida glabrata* (DSMZ
56 6425). This investigation could scientifically proof that the natural compounds of
57 *Chaetomorpha aerea* could be potentially used as antibacterial agents.

58 MATERIALS AND METHODS

59 Materials

60 Ground and dried *Chaetomorpha aerea* (Fig. 1), harvested on oyster ponds (Marennes-
61 Oléron, France) during winter (January 2009). Dowex Marathon C, BicinChonic Acid
62 (BCA) Protein Assay Kit, Azure A, N,O-bis(trimethylsilyl)trifluoroacetamide:
63 trimethylchlorosilane (BSTFA: TMCS) (99: 1), Zinc sulfate and Baryum hydroxide were
64 obtained from Sigma–Aldrich. Standard carbohydrates (dextran, dextran sulfatate, blue
65 dextran, heparin, fucoidan, glucose, galactose, rhamnose, fucose, fructose, xylose, arabinose,
66 mannose, lactose, raffinose, myo-inositol, glucuronic and galacturonic acid) and a protein
67 standard (Bovine Serum Albumin, BSA) were obtained from Sigma–Aldrich. Fucogel
68 1000PP, composed of a 3)- α -L-Fucp-(1 \rightarrow 3)- α -D-Galp-(1 \rightarrow 3)- α -D-GalpA-(1 \rightarrow
69 polysaccharide, was obtained from Solabia [19]. Solvents (chloroform, hexane, ethanol) were
70 from Carlo Erba. The ICSep ORH-801 and TSK Gel G3000 PWXL-CP columns for High

71 Performance Liquid Chromatography analysis (HPLC) were obtained from Interchim. The
72 DB-1701 J&W Scientific column (30m, 0.32mm, 1mm) for Gas Chromatography-Mass
73 Spectrometry analysis (GC/MS) was obtained from Agilent.

74 **Extraction and purification methodology**

75 From 5g of ground and dried *Chaetomorpha aerea*, a first step of delipidation and
76 depigmentation was performed by using mixtures of chloroform/hexane (2/1 v/v) (Fig. 2, Step
77 1). The second step consisted to extract the extracellular polymers through an aqueous
78 extraction procedure, during 24h at 40°C (Fig. 2, Step 2). A filtration stage was then applied
79 to clear out unwanted residues. After freeze-drying, the combination of aqueous solutions of
80 zinc sulfate (5%) and barium hydroxide (0.3N) on the sample allowed realizing the defecation
81 step (Fig. 2, Step 3). The supernatant was recovered after centrifugation then freeze-dried.
82 Finally, a purification step was applied to the sample through dialysis, to further purify the
83 carbohydrate fraction (Fig. 2, Step 4). This fraction was named *Chaetomorpha aerea*
84 carbohydrates-rich (CACR) fraction. A simple extraction was done (only the step 2) to
85 compare the composition of the CACR fraction with this water-extracted fraction. This
86 fraction was named control fraction (CF).

87 **Biochemical characterization**

88 Total sugar content was determined using the phenol-sulfuric acid assay, developed by
89 Dubois, using glucose as a standard [20]. Protein content was determined using the
90 bicinchoninic acid (BCA) assay, using bovine serum albumin (BSA) as a standard [21]. The
91 sulfate content was measured by the Azure A [22] and the barium chloride gelation method
92 [23], using dextran sulfate as a standard. Fourier Transform Infrared Spectroscopy (FTIR)
93 analyses were performed on the CACR fraction and commercial controls (dextran, dextran
94 sulfate, fucoidan, bovine serum albumin, Fucogel 1000PP) by using a Spectrum 100 FTIR

95 equipped with an Attenuated Total Reflectance (ATR) module and a crystal diamond.
96 Principal Component Analyses (PCA) were realized (XLStats) to characterize and classify the
97 IR spectrum of the CACR fraction among IR spectra of standards.

98 **Molecular weight determination**

99 Analysis of the carbohydrate fractions was carried out by HPLC using a Hewlett Packard
100 series 1100. The following conditions were used to determine the molecular weight M_x of the
101 polysaccharide by differential refractometry: 20 μ L of an aqueous solution of the purified
102 polysaccharides (10 to 50 g/L) were injected into a TSK Gel G3000 PWXL-CP column at
103 40°C, using water as elution solvent at a flow rate of 0.7mL/min. Dextran, dextran sulfate,
104 blue dextran, heparin, fucoidan, glucose, lactose, raffinose were used as standards.

105 **Carbohydrates monomers characterization**

106 Acidic hydrolysis conditions, i.e. 4h at 90°C in 2M HCl, were performed on purified fractions
107 to obtain samples containing mostly carbohydrates monomers. Preparations were then freeze-
108 dried and stored at 20°C. Prior to carbohydrates characterization by HPLC (Hewlett Packard
109 series 1100), 20 μ L of an aqueous solution of the fractions rich in monomers were injected
110 into a ICSep ORH-801 at room temperature, using an aqueous solution of H₂SO₄ 0.01M as
111 elution solvent at a flow rate of 0.6mL/min. Analysis of the hydrolyzed carbohydrate fractions
112 was also carried out by GC/MS using a Varian CP-3800 GC/Varian Saturn 2000. 400 mL of
113 pyridine and 400 mL of BSTFA: TMCS (99:1) was added to 2 mg of purified
114 polysaccharides. The solution was mixed for 2 h at room temperature, then injected into a
115 DB-1701 J&W Scientific column (30 m, 0.32 mm, 1 mm) at a flow of 1 mL/min. The helium
116 pressure was 8.8 psi. The temperature of the injector was set at 250°C. The rise in temperature
117 in the oven was programmed for a first step at 150°C for 0 min, then an increment of
118 10°C/min up to 200°C with a final step at 200°C for 35 min. The ionization was performed by

119 Electronic Impact (EI, 70 eV), the trap temperature was set at 150°C and the target ion was
120 fixed at 40–650 m/z.

121 **Microbiological material and microorganism sources**

122 Growth media were from Biokar Diagnostics and antibiotic solutions (ampicillin and
123 cycloheximid) were from Sigma-Aldrich. The microorganisms used in this study, i.e.
124 *Staphylococcus aureus* (ATCC 25923), *Salmonella enteritidis* (ATCC 13076), *Pseudomonas*
125 *aeruginosa* (ATCC 27853), *Enterococcus faecalis* (CIP 103214), *Bacillus subtilis* (CIP 5262),
126 *Micrococcus luteus* (ATCC 4698) and *Candida glabrata* (DSMZ 6425), were obtained from
127 American Type Culture Collection (ATCC), Collection of Institute Pasteur (CIP) and German
128 Collection of Microorganisms and Cell Cultures (DSMZ). All of the microorganisms were
129 provided in the form of pure bacterial stock culture.

130 **Antibiotic susceptibility testing**

131 Antimicrobial susceptibility testing was determined by the disc diffusion method (Sanofi,
132 Dianostics Pasteur). However, the method was adapted for the use of microwells, instead of
133 employing discs, which allowed working with larger volumes and concentrations. The
134 photometric calibration method was used to adjust the inoculum of the microbial suspensions:
135 microbial suspensions were prepared in a phosphate buffer (0.1M pH 7.2) adjusting the cell
136 density to $1-3 \cdot 10^8$ microbial cells/mL, corresponding to an optical density between 0.10 and
137 0.12 at 550nm. Microbial suspensions were then diluted in Mueller Hinton media (MH) to 1:
138 100. MH or Yeast Peptone Dextrose (YPD) agar plates (for *Candida glabrata*) were flooded
139 by these suspensions (2mL). After 15min at 37°C, sterile microwells (in glass, internal
140 diameter: 4mm, external diameter: 6mm, height: 7mm) were put onto the microbial field and
141 inoculated by 30µL of the CACR fraction. It is noteworthy that these samples were previously
142 re-solubilized at 20 and 50mg.mL⁻¹ in different solvents: methanol, acetone,

143 dimethylsulfoxide (DMSO) and ethanol. Ampicillin and cycloheximid (10mg/ml) were used
144 as positive controls. The plates were incubated at 30°C (for *Candida glabrata*) or 37°C and
145 the zones of inhibition were observed after 24h.

146 **Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)** 147 **tests**

148 The sensitivity of microorganism to CACR fractions was measured by using a tube dilution
149 technique, which allows the determination of the MIC and MBC of the seaweed used in this
150 study. These tests were done to determine the lowest concentration of the different extracts,
151 where the bactericidal and bacteriostatic effect can be shown. The test was performed in
152 tubes and allowed replicating each sample (triplicate). Microbial suspensions were prepared
153 in the same way than previously. Microbial suspensions, diluted in MH at 1: 100, were added
154 into increasing concentrations of CACR fractions, i.e. 2 to 50mg.mL⁻¹. The tubes were
155 incubated at 30°C or 37°C during 24h. The first clear tube before turbid samples allowed the
156 determination of the MIC. All clear tubes were placed out onto MH agar plates, and plates
157 were incubated at 30°C or 37°C for 24h. Finally, the number of microorganism colonies
158 developed on each agar plates was counted to determine the MBC, i.e. the plate where the
159 concentration of the CACR fraction was sufficient to destroy 99.99% of the microorganism
160 population. Ampicillin (10mg/ml) was used as positive control.

161 **RESULTS AND DISCUSSION**

162 **Biochemical characterization**

163 Many studies have highlighted that molecules from algae showed original biochemical
164 compositions, behind nutritional [24], medical and antibacterial properties [25]. Seaweeds
165 contain various compounds as polysaccharides, proteins, lipids, amino-acids, sterols or
166 phenolic molecules which show bioactivity against microorganisms [26, 27] or virus [28]. On

167 the other hand, sulfated polysaccharides extracted from marine algae can be used for their
168 anticoagulant and antithrombotic properties [29]. The main goal of this study was in a first
169 time to find a valorization path of the macroalga *Chaetomorpha aerea*, which is an ecological
170 problem for French west coast and especially oyster ponds. The extracellular polysaccharides
171 of this green alga were firstly extracted and their compositions were characterized. Indeed,
172 certain authors have highlighted that green algae as *Chaetomorpha* were composed of
173 interesting polysaccharides (arabinogalactans), sometimes sulfated [16, 18]. The biochemical
174 compositions (% w/w) of the control and the CACR fractions were determined (Table 1). The
175 control fraction (CF) was composed of carbohydrates (3.82%), proteins (2.30%) and other
176 compounds (93.9%) which were probably lipids, pigments and impurities. Owing to the
177 extraction procedure, the CACR fraction obtained was composed of 76.6% carbohydrates,
178 17.3% proteins and the unknown part was reduced to 5.8%. From 5g of *Chaetomorpha aerea*,
179 the method allowed the extraction of 233mg of the CACR fraction (extraction yield of
180 4.67%), whether approximately 178mg of carbohydrates and 40mg of proteins. The fraction
181 was rich in carbohydrates and proteins (17.3%) (Table 1), which was coherent with previous
182 works on a similar alga, *Chaetomorpha linum* [16]. It was noteworthy that the CACR fraction
183 contained a large part of sulfated carbohydrates (6.3%) (Table 1). Besides, carbohydrates
184 were sulfated (6.3%). In this way, Percival (1979) showed the important sulfatation degree of
185 polysaccharides extracted from *Chaetomorpha* and a sulfated polysaccharide (11.9%) has
186 been already purified from *Chaetomorpha antennina* [18].

187 FTIR analyses coupled to PCA allowed classifying the IR profile of the CACR fraction with
188 various IR profiles of commercial polymers (Fig. 3). The general composition of this fraction
189 was close to the composition of neutral and sulfated polysaccharides, as the dextran sulfate
190 (17% sulfur). Owing to its composition, the dextran sulfate is known for its anticoagulant
191 properties or its inhibitory effects against enzymes, cells or virus [30]. The general

192 composition and the sulfatation degree of the CACR fraction suggest that this extract could
193 present similar biological activities.

194 **Molecular weight determination and carbohydrates monomers characterization**

195 Gel permeation chromatography analyses allowed the determination of the molecular weight
196 of the CACR fraction. One peak was visible, at a retention time of 7.3min, by using the TSK
197 Gel G3000 PWXL-CP column (Fig. 4, A). Comparing this retention time to the logarithmic
198 standard curve, we concluded that the CACR fraction was composed of a main
199 polysaccharide of $1.160 \times 10^6 \pm 0.150 \times 10^6$ Da. Other HPLC analyses, realized by using the
200 ICSep ORH-801 fraction, showed that the CACR fraction was composed of three main
201 monosaccharides: glucose, galactose and arabinose (Fig. 4, B). CPG/MS analyses confirmed
202 the presence of glucose (24%), galactose (58%), arabinose (18%) and the traces of xylose
203 (Table 2). The homology of MS spectra was verified (>91%), by comparing the MS spectra of
204 standards and the MS spectra of the monosaccharides identified in the CACR fraction.
205 According to previous studies [16, 18], the identification of a xyloarabinogalactan from the
206 extracellular polysaccharides of a green alga was coherent and interesting.
207 Xyloarabinogalactans, water-solubles and extracted from *Chlorophyceae*, are branched and
208 sulfated heteropolysaccharides, presenting various composition, without repeating unit, except
209 residues of (1,4)-L-arabinose separated by D-galactose units [16]. Although it is difficult to
210 characterize the complete structure of this type of sulfated heteroglycans, numerous studies
211 have highlighted the anti-herpetic, anti-coagulant activities or antioxidant capacity of sulfated
212 galactans extracted from seaweeds [31].

213 **Antibiotic susceptibility testing**

214 The second part of this work was dedicated to a preliminary screening of the potential
215 antibacterial activities of these natural extracted polysaccharides against various strains of

216 microorganisms, including pathogenic or resistant bacteria (*Pseudomonas aeruginosa*,
217 *Staphylococcus aureus*), which contaminate many biological or inert surfaces. Inhibition
218 zones were measured for each re-suspended CACR fractions ($50\text{mg}\cdot\text{mL}^{-1}$). Positive controls
219 confirmed that the strain correctly grew up and was affected by ampicillin. Negative controls
220 showed that the solvents used did not affect the growth of the different microorganisms. First,
221 no inhibition area was observed for the strains *Salmonella enteritidis* (ATCC 13076),
222 *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (CIP 103214) or *Candida*
223 *glabrata* (DSMZ 6425) (Table 3). Only the strains *Bacillus subtilis* (CIP 5262), *Micrococcus*
224 *luteus* (ATCC 4698) and *Staphylococcus aureus* (ATCC 25923) were affected by the presence
225 of the CACR fraction resuspended in water (Table 3). Moreover, the strain of *Staphylococcus*
226 *aureus* (ATCC 25923) was affected by different CACR fractions, re-suspended in different
227 solvents (Fig. 5). *Staphylococcus aureus* (ATCC 25923) showed an important sensibility to
228 the CACR fractions during the contact periods (Fig. 5). The methanol CACR extract showed
229 the greatest diameter of inhibition, i.e. $13\text{mm} \pm 1$ (Fig. 5, D; Table 4), probably due to a better
230 solubility of the molecules composing the CACR fraction (Table 4). It is important to note
231 that the CACR fractions had an inhibitory activity only against three gram-positive
232 microorganisms. Antimicrobial activities from seaweeds are mostly higher recurrent against
233 gram positive bacteria as *Staphylococcus aureus* [32]. However, no inhibitory activity of the
234 CACR fraction was found against *Enterococcus faecalis* (CIP 103214). Several authors
235 clarified there is several reasons to explain why biological extracts could be active or not
236 against different microbial strains, e.g. (i) the absence of target structure in the bacteria, (ii)
237 the cell wall of the bacteria or (iii) the ability of the bacteria to modify the structure of the
238 molecules composing the tested fraction [33].

239 **MIC and MBC tests for the pathogenic strain *Staphylococcus aureus* (ATCC 25923)**

240 Bacterial turbidities of *Staphylococcus aureus* (ATCC 25923) in contact with increasing
241 concentrations of the CACR fraction (re-suspended in water or methanol) allowed the
242 determination of the MIC and MBC. No bacterial turbidity, corresponding to the MIC values,
243 was found at 40 and 42 mg.mL⁻¹ for the methanol and water CACR fractions respectively
244 (Table 4). This result indicated that the CACR fraction could block the cell wall formation of
245 this bacterium, inducing its lysis and death. However, it is noteworthy that the concentration
246 of the antibacterial agent can greatly influence its classification as bacteriostatic or
247 bactericidal agent. The protocol used to determine the MBC of the methanol and water CACR
248 fractions highlighted a MBC value of 45 mg.mL⁻¹ for both. The MBC/MIC ratio indicated
249 that the two CACR fractions presented a bactericidal activity against *Staphylococcus aureus*
250 (ATCC 25923). However, a high dose of a bacteriostatic antibacterial molecule (as 45
251 mg.mL⁻¹) will be bactericidal [12]. Finally, several significant findings were found whereby
252 the CACR fraction, composed of a sulfated xyloarabinogalactan, which exhibited a selective
253 inhibitory activity against the Gram-positive bacterium *Staphylococcus aureus* (ATCC
254 25923). *Staphylococcus aureus* is a common pathogen spread by ingestion of contaminated
255 food or water. Seafood is one of the sources of staphylococcal infection for humans [34].
256 Moreover, marine animals, as oysters, may be reservoirs or carriers of infectious agents and
257 biological toxins [35]. In this way, studies have highlighted that oysters could be
258 contaminated by *Staphylococcus aureus*, indirectly from contaminated water [36, 37]. The use
259 of *Chaetomorpha aerea* in oyster ponds could be allowed to biologically purify water. The
260 “secretion” of its extracellular sulfated galactan could avoid the microbial contamination of
261 oysters against this pathogen bacterium, and finally could minimize the number of human
262 food poisoning.

263 **CONCLUSION**

264 Therefore, this study allowed the extraction and partially characterization of an extracellular
265 polysaccharide from the green alga *Chaetomorpha aerea*. The CACR fraction was mainly
266 composed of one type of polysaccharide of 1.160×10^6 Da. This fraction contained 76.6%
267 carbohydrates and 6.3% of them were sulfated. Chromatographic and GC/MS analyses
268 highlighted that the polysaccharide is a xyloarabinogalactan, composed of 17.9% arabinose,
269 23.8% glucose, 58.3% galactose and some traces of xylose. Microbiological tests showed that
270 the CACR fraction selectively inhibited the growth of *Staphylococcus aureus* (ATCC 25923)
271 and could be potentially a bactericidal agent ($40 \text{mg} \cdot \text{mL}^{-1}$). It could be interesting to better
272 purify the CACR fraction by using ion exchange resins to eliminate the presence of proteins.
273 On the other hand, the fact that the CACR fraction was composed of a sulfated
274 xyloarabinogalactan is of primary interest since these kinds of algal sulfated heteroglycans are
275 known for their biological activities as antimicrobial and especially anti-coagulant properties.
276 The presence of this kind of polysaccharide could be a potential path to valorize the
277 deleterious alga *Chaetomorpha aerea*.

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375 contamination and purification of bivalve shellfish: crucial aspects in monitoring and future
376 perspectives – a mini-review. Food Control. 22: 805-816.

377

378 Table 1. Biochemical composition (% w/w) of the native sample and the CACR fraction
 379 extracted from *Chaetomorpha aerea*.

Composition (% w/w)	Carbohydrates	Uronic acids	Proteins	Sulfated carbohydrates	Others
Control fraction	3.82 ± 0.12	nd	2.30 ± 0.11	0.33 ± 0.14	93.9
Partially purified fraction	28.1 ± 2.31	nd	6.53 ± 1.08	2.21 ± 0.92	71.9
CACR fraction	76.6 ± 0.62	nd	17.3 ± 2.85	6.3 ± 1.2	5.8

380 *nd: non determined by colorimetric assays*

381 \pm : *Standard deviation σ_x on 10 runs.*

382

383 Table 2. Identification and quantification by GC/MS of the monosaccharides composing the
 384 CACR fraction extracted from *Chaetomorpha aerea*.

Monosaccharides	Retention time of standards	Retention time of peaks in the CA fraction	Homology MS spectra (%)	Concentration (% w/w)
Rhamnose	6.49	-	-	-
Arabinose	6.61	6.59	93.45	18
Fucose	7.65	-	-	-
Xylose	8.59	9.11	91.87	trace
Mannose	10.72	-	-	-
Galactose	11.73	11.72	98.12	58
Glucose	12.67	12.59	98.89	24
Inositol	23.89	-	-	-

385

386

387 Table 3. Antibacterial activities against the different gram-positive/gram-negative bacteria of
388 the CACR extract, previously re-solubilized in water.

Microorganisms	<i>Diameters of inhibition (mm)</i>
<i>Bacillus subtilis</i> (CIP 5262)	14 ± 2
<i>Micrococcus luteus</i> (ATCC 4698)	13 ± 2
<i>Staphylococcus aureus</i> (ATCC 25923)	11 ± 0.5
<i>Enterococcus faecalis</i> (CIP 103214)	0
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	0
<i>Salmonella enteritidis</i> (ATCC 13076)	0
<i>Candida glabrata</i> (DSMZ 6425)	0
Ampicillin (positive control)	41 ± 1.5

389 ±: Standard deviation σ_x on 10 runs.

390

391

392 Table 4. Antibacterial activities of the CACR extract against *Staphylococcus aureus* (ATCC
393 25923), previously re-solubilized in different solvents.

Solvents used to solubilize extracts	<i>Diameters of inhibition (mm)</i>
Water (50mg.mL⁻¹)	11 ± 0.5
Ethanol (50mg.mL⁻¹)	11 ± 1
Acetone (50mg.mL⁻¹)	12 ± 0.5
Methanol (50mg.mL⁻¹)	13 ± 1
Dimethylsulfoxyde (50mg.mL⁻¹)	10 ± 0.5
Ampicillin (10mg.mL⁻¹)	41 ± 1.5

394 ±: Standard deviation σ_x on 10 runs.

395

396

397 Table 5. Bacterial turbidity of *Staphylococcus aureus* (ATCC 25923) after 24h at 37°C in
 398 contact with different concentrations of the CACR extract, previously re-suspended in water
 399 or methanol. Ampicillin was used as positive control.

Concentrations (mg.mL ⁻¹)	Ampicillin	CACR water-fractions	CACR methanol-fractions
2	-	+++	+++
4	-	+++	+++
8	-	+++	+++
10	-	+++	+++
15	-	+++	+++
20	-	+++	+++
30	-	++	++
35	-	+	+
40	-	+	-
42	-	-	-
45	-	-	-
50	-	-	-

+++ : very turbid microbial suspension

++: turbid microbial suspension

+: low turbid microbial suspension

-: no turbidity

400

401

402 Figure 1. *Chaetomorpha aerea*, the type of green alga which was used in this study.

403 Figure 2. Extraction and purification procedure which was developed to obtain the CACR
404 fraction from the mucilage of *Chaetomorpha aerea*.

405 Figure 3. Principal Component Analysis (PCA) (XLStat) of the FTIR spectra obtained for the
406 CACR fraction and different commercial polysaccharides and proteins. The spectral region
407 selected for the PCA analysis was comprised between 650 and 4000 cm^{-1} .

408 Figure 4. Analyses by HPLC: determination of the molecular weight by gel permeation
409 chromatography (A) and characterization of the monosaccharides distribution (B) of the
410 polymer composing the CACR fraction.

411 Figure 5. Sensibility of *Staphylococcus aureus* (ATCC 25923), after 24h at 37°C, against
412 different re-suspended CACR fraction (50 $\text{mg}\cdot\text{mL}^{-1}$): water (A), ethanol (B), acetone (C) and
413 methanol (D) CACR fractions. T+ corresponded to the sensibility of the strain against
414 ampicillin. T- showed the non-effect of the solvent used on the strain growth.

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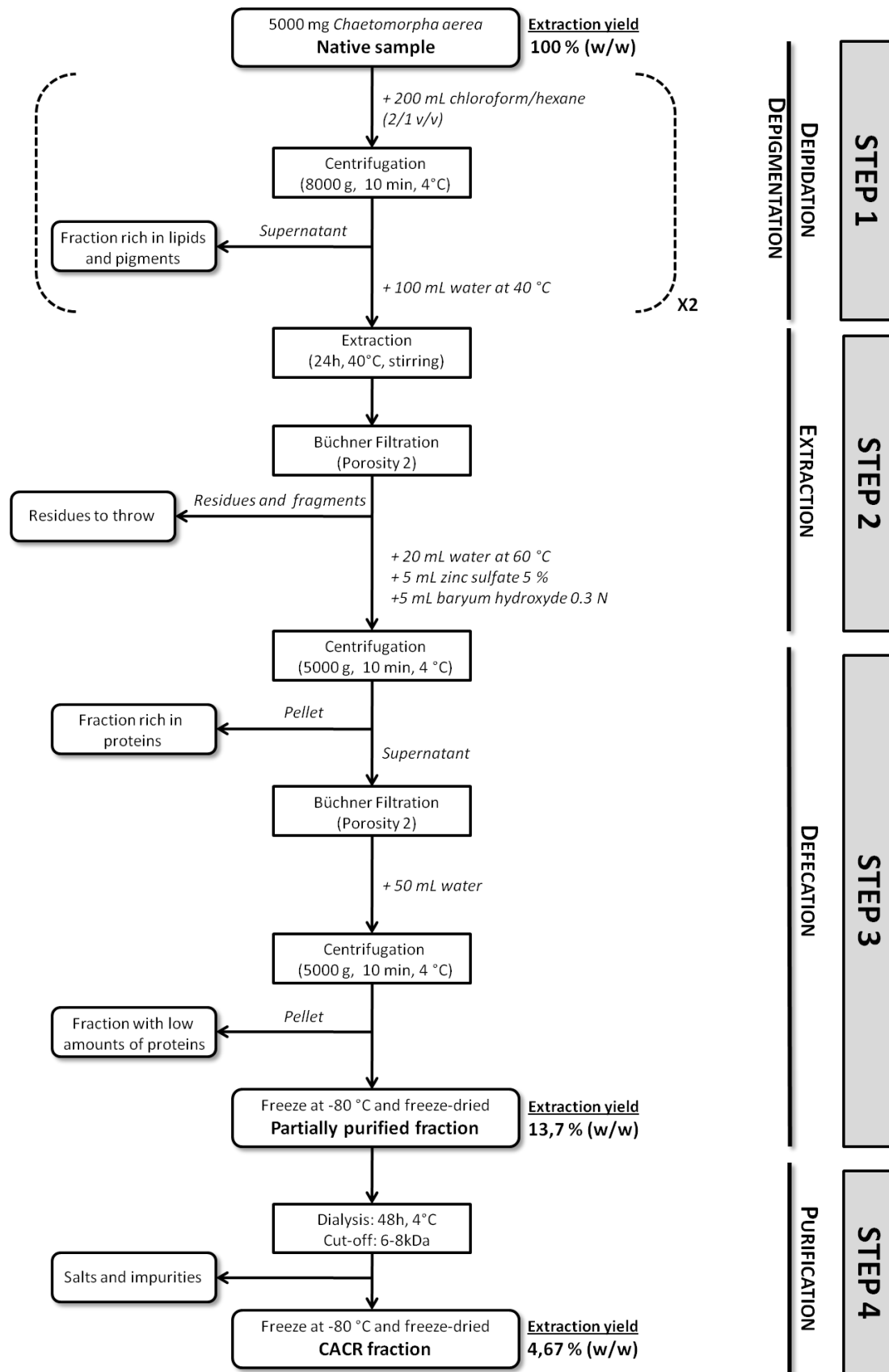
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FIGURE 1

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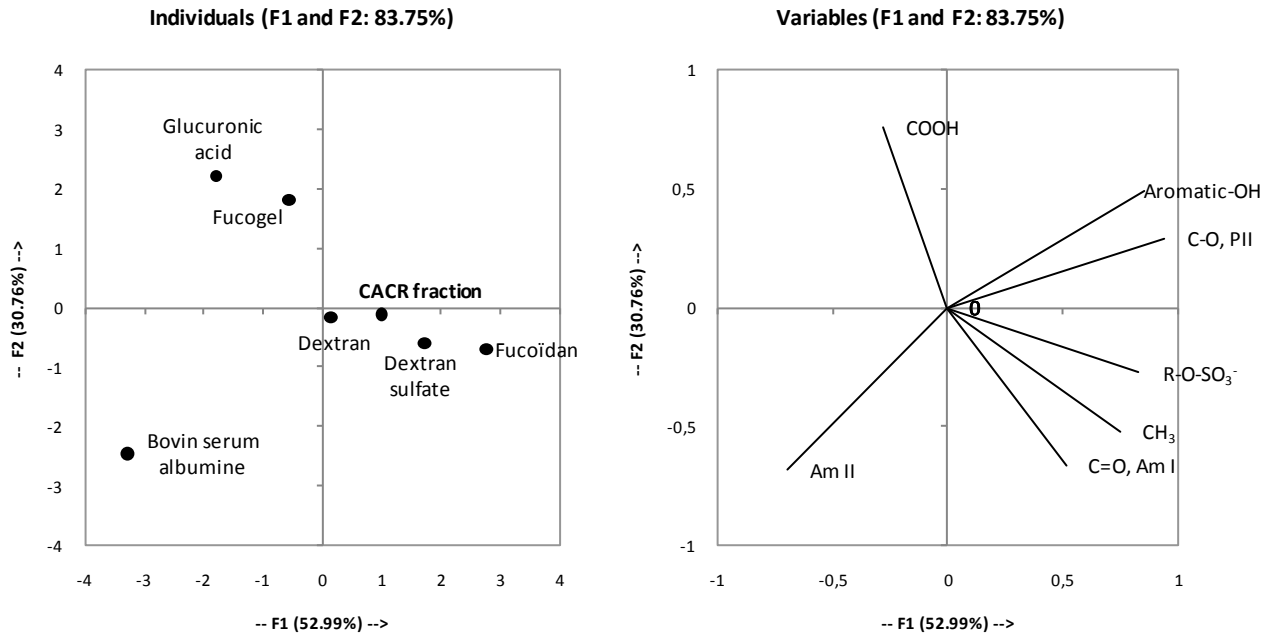


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FIGURE 2



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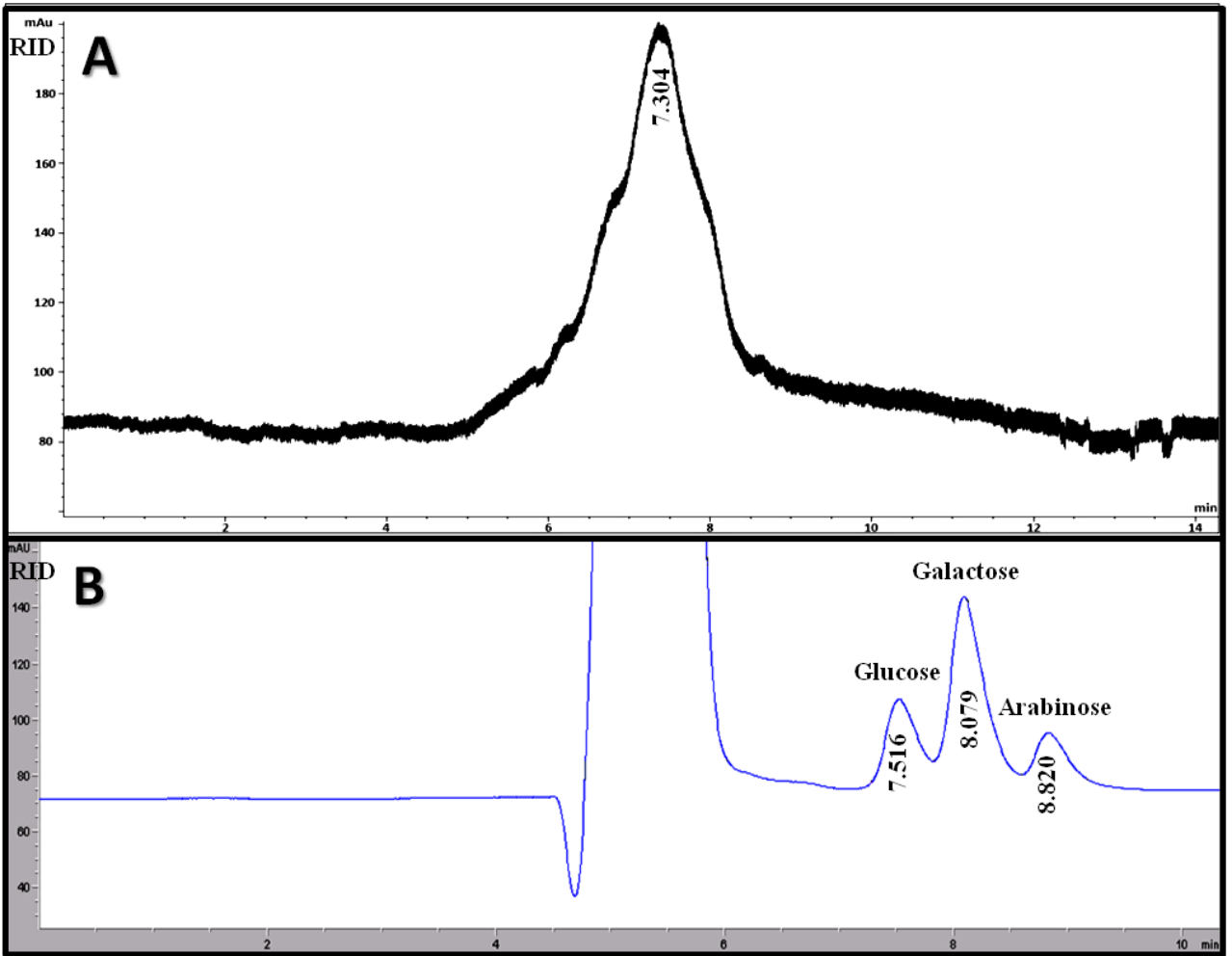
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FIGURE 3



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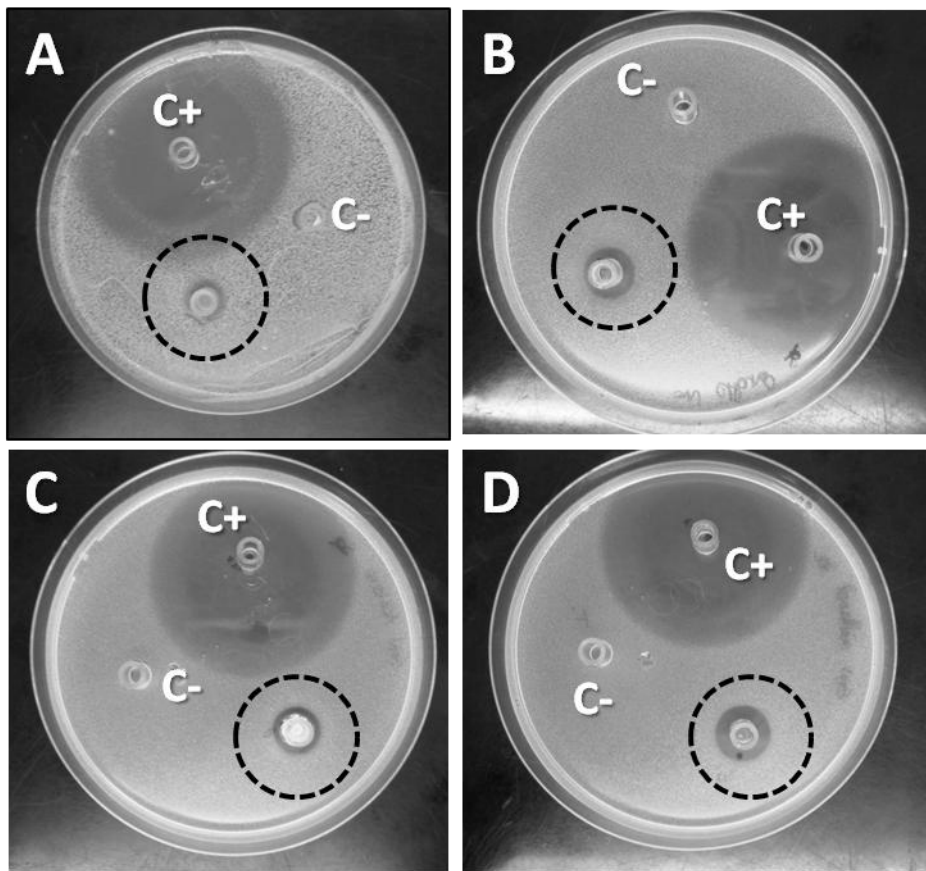
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FIGURE 4



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FIGURE 5