ANTIMICROBIAL PROPERTIES OF SOME ALGAL DERIVED BIOPRODUCTS

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Algae represent a valuable raw material currently used in food, pharmaceutical, and cosmetic applications. In this study, four algal species, respectively P. umbillicalis, U. pinnatifida, C. barbata and Chlorella sp. were utilized to obtain two types of bioproducts (eight bioproducts in total) derived from aqueous or alcoholic extracts of each species. The content of polyphenolic compounds was quantified for each standardized bioproduct, and their antimicrobial activities were assessed using eight human pathogenic microorganisms. The results revealed local or moderate antimicrobial activities against S. aureus, S. aureus MRSA, P. aeruginosa, S. typhimurium, E. coli, S. marcescens, C. albicans, and C. parapsilosis. In conclusion, the bioproducts obtained from alcoholic extraction can represent potentially antimicrobial reagents, but more studies are needed.

Keywords: algal bioproducts, human pathogens, antimicrobial properties

1. Introduction

Sessile marine organisms, such as seaweeds, have adapted to environmental conditions to survive by developing specific defence strategies. These strategies involve the biosynthesis of secondary metabolites, such as laminarins, fucoidans (algal polysaccharides), and phlorotannins (polyphenolic compounds specific to seaweeds, which are polymers derived from phloroglucinol and may contain halogenated derivatives such as bromophenols, phenolic acids, pigments, etc.) [1]. Currently, the most widely utilised algae include *Porphyra umbilicalis, Undaria pinnatifida*, *Cystoseira barbata*, and *Chlorella sp*., due to their applications in the food, cosmetic, and agricultural industries [2–5]. In 2023, algae farms produced approximately 2,017,000 tonnes of *Porphyra umbilicalis* and 2,300,000 tonnes of *Undaria pinnatifida* [2]. These algae are used either in dry form or as extracts containing polysaccharides, which are added to food products in proportions not exceeding 3%. For instance, algae are included in products such as beef, pork, or

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various types of cheese, primarily to improve the sensory properties of the final products [1]. Additionally, bakery products and animal products (e.g., meat, yoghurt) enriched with algae additives are rich in fibres with prebiotic or antioxidant roles [6–10]. Some of the compounds biosynthesised by the algae from the orders Rhodophyta (red algae), Chlorophyta (green algae), Fucales (brown algae), or Laminariales possess antimicrobial properties [11–15] against pathogenic and/or phytopathogenic bacteria, yeasts, or fungi [15; 16–18]. The compounds involved in these activities are most often polyphenolic compounds specific to algae, such as fucophloretols and phlorotannins [21–24], or halogenated phlorotannins, including bromophenols [25–28] and phenolic acids [29–32] (Fig. 1). Biologically active molecules produced by micro- or macroalgae are typically obtained through classical extraction methods using water, alcohols, acetone, or ethyl acetate. Alternatively, they can be extracted using modern techniques, such as: extraction with supercritical fluids, microwave-assisted extraction, or ultrasound-assisted extraction [11, 13, 14, 21, 22, 24]. Studies on the antimicrobial activity of alcoholic extracts from *Cystoseira barbata* (Fig. 2) have shown that these extra*cts* generally exhibit local antimicrobial activities against most microorganisms such as *Staphylococcus aureus*, *Staphylococcus. aureus MRSA*, *Escherichia coli, Candida sp., Pseudomonas sp*., and *Salmonella typhimurium* [33, 34].

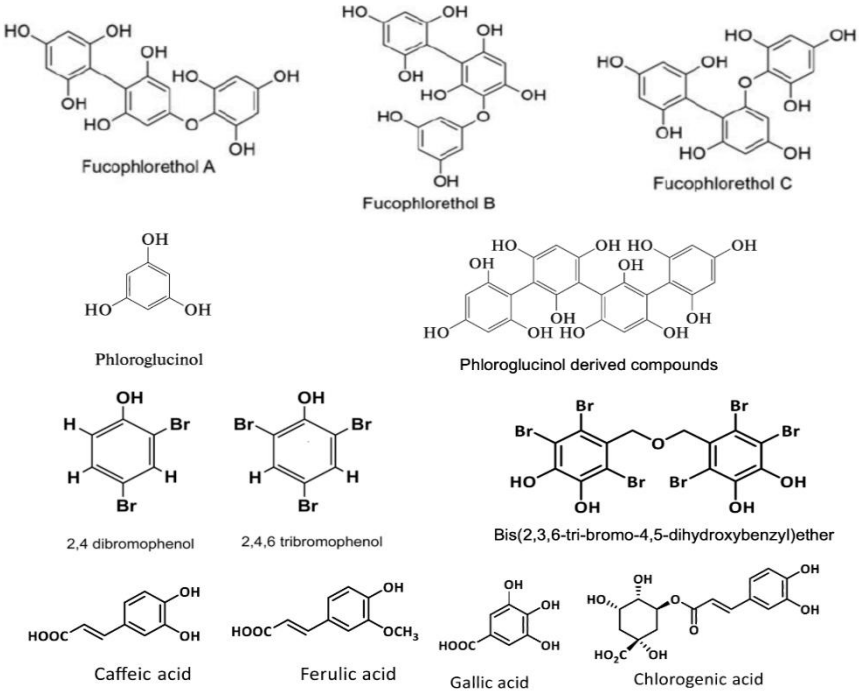


Fig. 1. Basic structure of some phlorotannines, bromphenols an phenolic acids identified in studied algae [22; 28-29; 32].

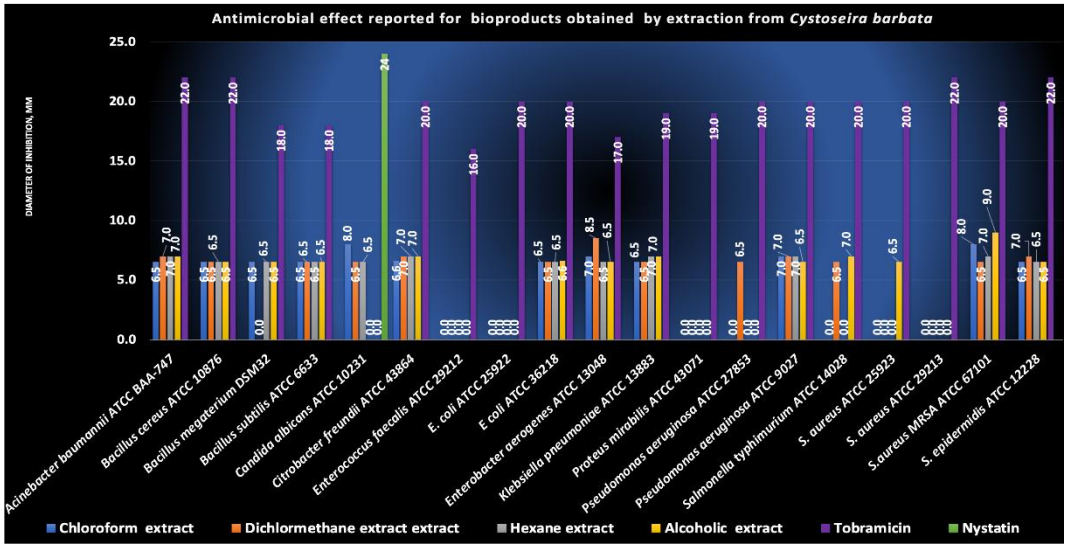
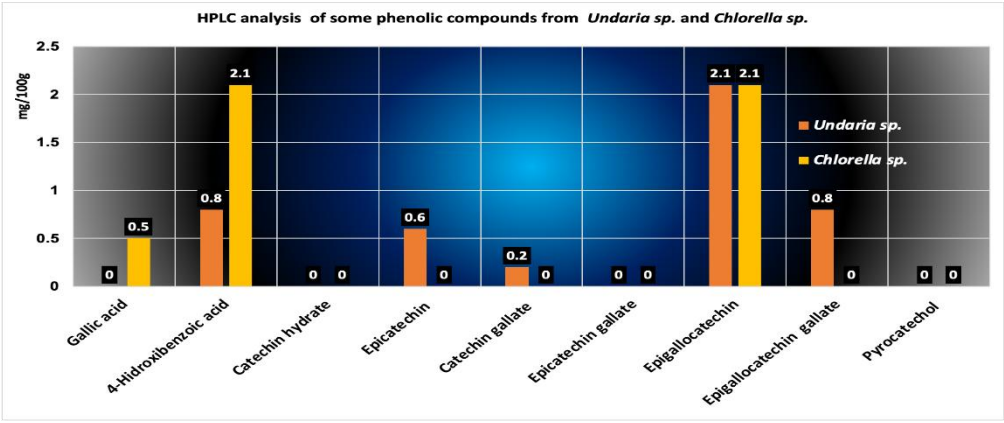


Fig. 2. Antimicrobial effect of different bioproducts obtained by extraction from *C.barbata* and different solvents (adaptation after Kamaran and colab. [33]).

Undaria pinnatifida, a brown algae species also widely used in the food industry, as well as *Cystoseira barbata*, (an endemic brown algae) contains various classes of polyphenolic compounds (Fig. 3a,b) such as:

- phlorotannins (compounds specific to brown algae, polymers derived from phloroglucinol, playing a role in protection against oxidative stress)
- eckols (polyphenols from the phlorotannin category, phloroglucinol derivatives formed by the condensation of multiple phenolic units), including eckol, a compound with a triphenolic structure, dimers of eckol such as dieckol, bieckol, fucodiphloroethol, phlorofucofuroeckol A, *Chlorella sp*., a green microalgae, may contain the following classes of polyphenols (Fig. 3a): flavonoids (quercetin, kaempferol, rutin); phenolic acids (chlorogenic acid, caffeic acid, ferulic acid, gallic acid); tannins; lignans; catechins (epicatechin, epigallocatechin gallate). *Porphyra umbilicalis*, a species of red algae widely used in the food industry, contains several classes of polyphenols [21-24, 34-36] (Fig. 3c), namely:
- flavonoids (epicatechin, epigallocatechin, quercetin, kaempferol);
- phenolic acids (gallic acid, ferulic acid, caffeic acid, p-coumaric acid);
- tannins (phlorotannins, monomers, oligomers, or polymers of phloroglucinol);
- phycobilins (pigments with a polyphenolic structure, known as phycocyanin and allophycocyanin);
- lignans (phenylpropanoid units linked via β-β' bonds, forming dimers or more complex structures).

Regarding the content of polyphenolic compounds, the content reported indicated a content of up to 100mg/100 g bioproduct (Fig. 3 a,b,c) [34-36].



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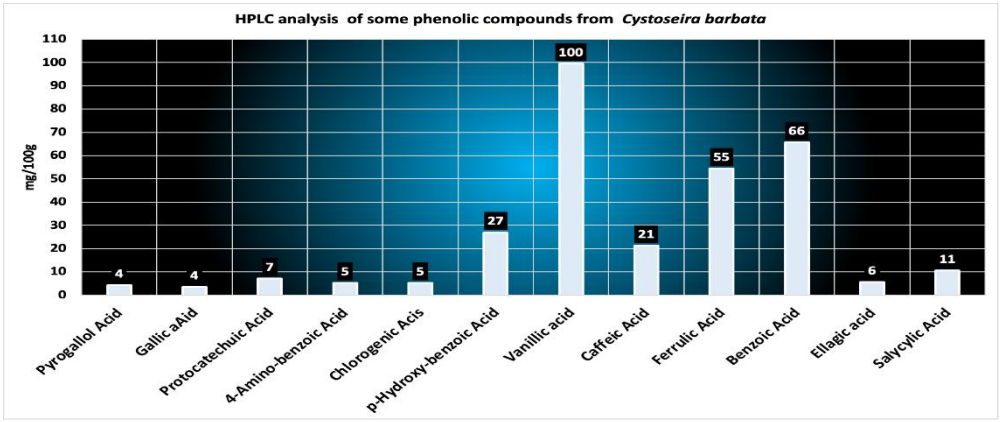

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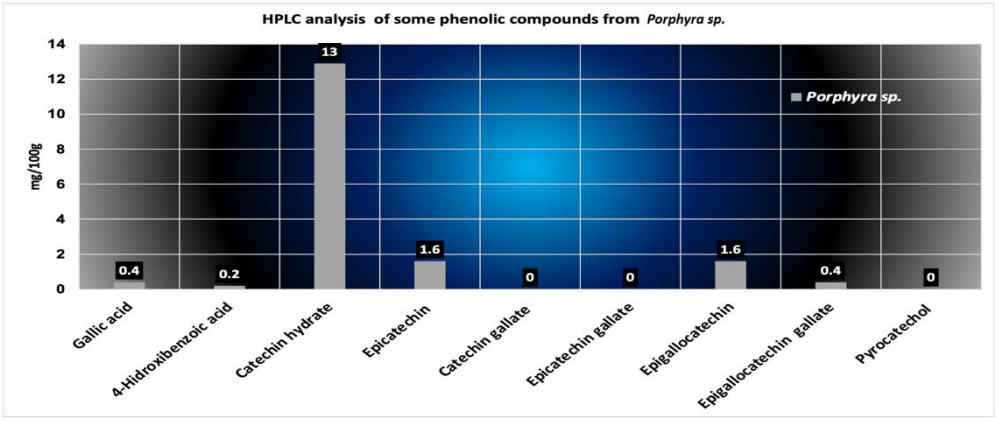

c

Fig. 3. Phenolic compound concentrations reported for: a) *Chlorella sp*, and *Undaria pinnatifida* (adaptation after Machi et al. [36]); b) *Porphyra umbilicalis, Undaria pinnatifida* (adaptation after Machi et al. [36]); c) *Cystoseira barbata* (adaptation after Cadar et al, [34]).

The studies performed aimed to evaluate the antimicrobial properties of two types of biomaterials obtained through extraction with selective solvents (water followed by ethanol) by refluxing in aqueous and/or alcoholic media from four species of algae.:

- *Porphyra umbilicalis;*
- *Undaria pinnatifida*;
- *Cystoseira barbata*;
- *Chlorella sp.*

2. Materials and methods

2.1. Algal bioproduct

Four algal species were used to obtain crude polysaccharide and polyphenol extracts, following the methodology illustrated in Figs. 4a and 4b. The algal biomass for *Porphyra umbilicalis* and *Undaria pinnatifida* was sourced from Romanian markets, while *Cystoseira barbata* was provided by Ovidius University of Constanta Romania (specimens recovered during studies regarding flora and fauna from the Black Sea).

Biomass of *Chlorella sp*. was supplied by National Institute of Chemistry and Petrochemistry R&D (INCDCP) of Bucharest of Bucharest Romania, which cultivated the species in a photobioreactor. All bioproducts are used in the current studies as standardized solution in Dimethyl Sulfoxide (DMSO) 20% (A1; A2; A3; A4) and respectively in Ethanol 40% (A5; A6; A7; A8).

In this way (Fig. 4 a, b), were obtained eight bioproducts, from which four bioproducts with polysaccharides, named:

A1 (polysaccharides enriched extract from *Porphyra umbilicalis*),

A2 (polysaccharides enriched extract from *Undaria pinnatifida*),

A3 (polysaccharides enriched extract from *Cystoseira barbata*),

A4 (polysaccharides enriched extract from *Chlorella sp*.) and four bioproducts with polyphenols, named

A5 (polyphenolic enriched extract from *Porphyra umbilicalis*,

A6 (polyphenolic enriched extract from *Undaria pinnatifid*a),

A7 (polyphenolic enriched extract from *Cystoseira barbata*),

A8 (polyphenolic enriched extract from *Chlorella sp*.).

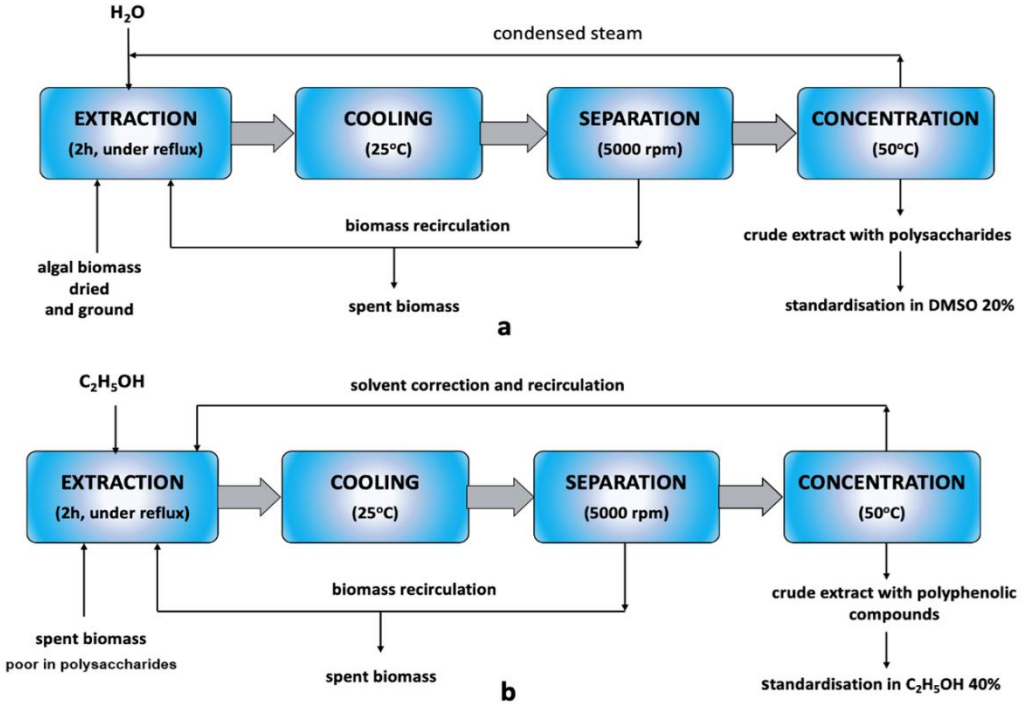

Fig. 4. Flow charts used to obtain algae bioproducts: a) bioproducts enriched with polysaccharides; b) bioproducts enriched with polyphenolic compounds. Source: Own studies

2.2. Antimicrobial properties

Antimicrobial properties were assessed using the Kirby-Bauer diffusion method, using Mueller-Hinton Agar culture media (Merck, Bucharest, Romania) and 90 mm Petri plates. A total of eight microbial strains were tested as follows: *Candida albicans* ATCC 10231; *Candida parapsilosis* ATCC 22019; *Staphylococcus aureus* ATCC 25923; *Staphylococcus aureus* MRSA ATCC 33592; *Serratia marcescens* ATCC 14756; *Pseudomonas aeruginosa* ATCC 13388; *Escherichia coli* ATCC 11303; *Salmonella enterica* ATCC 51741.

All strains were previously activated by inoculating each microorganism from lyophilised loops onto Petri dishes with Tryptic Soy Agar culture media (Merck, Bucharest, Romania) for bacteria and Potato Dextrose Agar (Merck, Bucharest, Romania) for *Candida species*. After microbial growth, a suspension of each microorganism was prepared under sterile conditions and adjusted to a turbidity corresponding to the 0.5 McFarland standard. Each Petri dish containing Mueller-Hinton medium was then inoculated using a sterile cotton swab.

Afterwards, four sterile paper discs, each with a diameter of 6 mm, were introduced into each Petri dish. These discs were impregnated with 30 µL of each bioproduct.

Each bioproduct used in this test was previously sterilized by passing it through a 0.2-micron membrane filter (Solantis, Bucharest, Romania). Separately, standardized antibiotic discs (Bio-Rad Laboratories, Hercules, CA, USA) with Clotrimazole (CT10), Ampicillin (AMP25), Tobramycin (TOB10), Chloramphenicol (C30), Minocycline (MIN 2.5 and MIN 5), Piperacillin (PI100), Imipenem (IPM10), Piperacillin-Tazobactam (PIT100/10), Gentamicin (GEN 10), Ticarcillin/Clavulanate (TCC75/10), Amoxicillin (AX10), and Fosfomycin (FO200), specific to each microorganism tested, were subjected to the same procedure.

All Petri dishes were incubated for 24 hours at 37°C for bacteria and 48 hours at 37°C for *Candida albicans*. After the incubation period, the inhibition diameters were measured. The inhibition diameter was measured for at least three tests, and the results were presented as mean values along with their standard deviations.

2.3. Polyphenols content evaluation from the bioproducts derived from algal basis

A volume of 1.5 mL of distilled water was carefully mixed with 100 µL of Folin-Ciocalteu reagent (Merck, Bucharest, Romania), followed by adding 100 µL of the algal extract and 300 µL of 20% sodium carbonate (Na_2CO_3) aqueous solution.

The components were introduced sequentially, in the specified order, ensuring thorough mixing after each addition. The resulting mixture was left to stay in the dark for 120 minutes. After 120 minutes, the absorbance of each sample was measured spectrophotometrically at 765 nm, with distilled water as the reference.

The obtained results were expressed in terms of milligrams of Gallic Acid Equivalents per litre (mg GAE/L). All physicochemical tests were conducted in triplicate, and the results were expressed as mean values accompanied by their standard deviations.

2.4. Device

Microbiological hoods type Bio48 Faster (Cornaredo, Italy), incubator LbX (LbX Instruments, Barcelona, Spain), rotary vapor type Heidolph, (Schwabach, Germany), centrifuge Hettich 32R (Hettich GmbH & Co, Tuttlingen, Germany), UV-VIS spectrophotometer type Evolution 220, (Termo Fisher Scientific, Leicestershire, UK).

2.5. Other reagents

Distilled water (sterile); Dimethyl sulfoxide (DMSO) (Merck, Bucharest Romania), ethanol 98% (Merck, Bucharest Romania).

3. Results and discussions

Regarding the polyphenol content of the eight obtained bioproducts (Fig. 5), the highest value was observed for sample A3 (714 mg GAE/L), derived from the aqueous extract of *Cystoseira barbata*. This was followed by sample A4, derived from the aqueous extract of Chlorella sp. (55 mg GAE/L). The bioproducts obtained from the alcoholic extracts of *Chlorella sp*. and *Cystoseira barbata* (A8) and the aqueous extract of *Porphyra umbilicalis* contain the same level of polyphenolic compounds (20 mg GAE/L). The alcoholic extracts A6, A7, and A5 contained 15 mg GAE/L, 10 mg GAE/L, and 7 mg GAE/L, respectively.

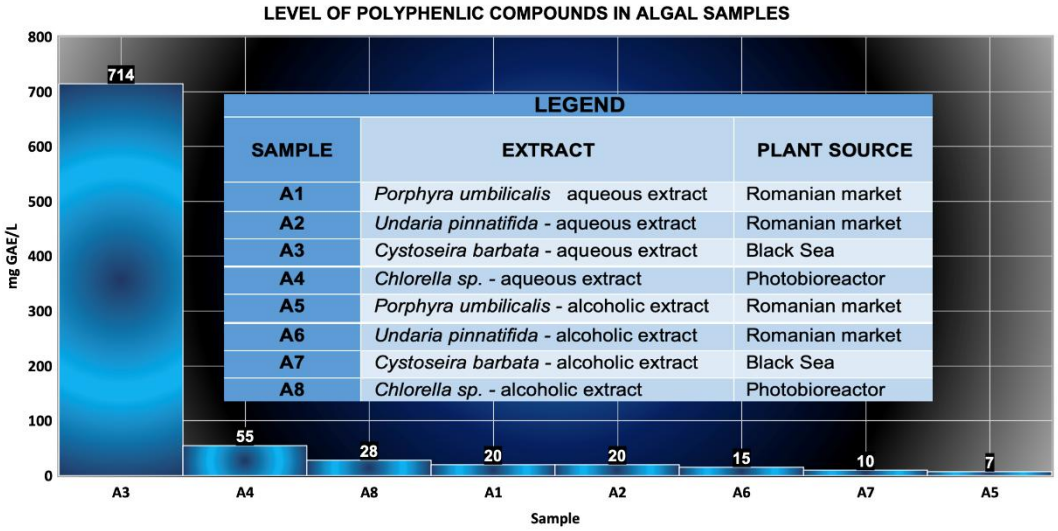

Fig. 5. Polyphenolic compound content in algal bioproducts. Source: Own studies

These results are in agreement with studies performed by other scientists. Cadar et al. [34] have reported a polyphenol content of 386 mg GAE/100 g for *Cystoseira barbata* harvested from the Black Sea, while Kosanic et al. [35] reported for a similar bioproduct obtained from *C. barbata* harvested from the Adriatic Sea, a polyphenol content of 62 mg GAE/L. Machu et al. [36] reported polyphenols content in aqueous or alcoholic extracts derived from three algal species, of (5.9 - 8.6*)* mgGAE/g *for Undaria pinnatifida,* respectively of (15–18 mg*)GAE/g for Porphyra sp.,* and 18 mg GAE/g for bioproducts obtained from *Chlorella sp.* Other researchers [34, 36] have analysed the phenolic compound content of similar bioproducts derived from the same algal species using HPLC techniques. They found content of 2.1 mg/100 g of epigallocatechin in *Undaria sp.* and *Chlorella sp.* 13mg/100 g of catechin hydrate in *Porphyra sp.*, and 100 mg/100 g of vanillic acid in *Cystoseira barbata*. Studies on the antimicrobial activities of eight bioproducts against gram-positive bacteria (Fig. 6 a,b,c) reveal the following:

- bioproducts obtained by aqueous extraction do not exhibit antimicrobial properties; only bioproducts A5 and A8 show moderate antimicrobial activity against *Staphylococcus aureus* (Fig. 6a) for which the inhibition diameters of 17 mm and 12 mm were obtained. These findings align with those of other researchers [1, 33], who reported antimicrobial properties of phlorotannins and halogenated bromophenols isolated from red algae [1].

Additionally, they are consistent with the antimicrobial activity of alcoholic extracts obtained from *Chlorella sp.* against *Staphylococcus aureus* [37], for which an inhibition diameter of 11 mm was reported [37]. The observed antimicrobial activities can be attributed to specific algal polyphenols of these species (Table 1), (*Porphyra umbilicalis* contains fucophlorethols and phloroglucinol-derived compounds (phlorotannin polymers) [21, 22]; *Chlorella sp.* contains polyphenols such as caffeic acid, ferulic acid, gallic acid, p-coumaric acid, chlorogenic acid, 4-hydroxybenzoic acid and epigallocatechin [30–32; 36]);

-in the case of *Staphylococcus aureus MRSA* (Fig. 6b), bioproducts A5 and A8 exhibit moderate antimicrobial effects, with both yielding an inhibition diameter of 10 mm. The results obtained for crude extracts derived from *Chlorella sp.* are supported by Shaima et al. [37], who reported an inhibition diameter of 14 mm for a bioproduct obtained via alcoholic extraction containing 2g/L crude extract.

Table 1.

Specific polyphenolic compounds and their biological properties reported for the *studied algae*

Nr. crt	Species	Phlorotannines/ Bromphenols/ Phenolic acids content	Biologic properties	References
0	1	2	3	4
1	*Porphyra umbilicallis**	Fucophlorethols Phloroglucinol derived compounds (phlorotannin polymers	Antioxidant antiinflamatories	[21; 22]
		Probably Bromophenols*	Antimicrobial properties	
2.	*Undaria pinnatifida*	Fucophlorethols; Phloroglucinol-derived compounds; (phlorotannin oligomers and polymers)	Antioxidant Antiinflammatory Antimicrobial Anticancer	[23; 24]
		2,4-dibromophenol 2,6-dibromophenol 2,4,6-tribromophenol	Antioxidant Antimicrobial	[25]
0	1	2	3	4
3.	*Cystoseira barbata*	Fucophlorethols Phloroglucinol-derived compounds (phlorotannins polymers)	Antioxidant Antiinflammatory Antimicrobial Anticancer	[26; 27; 28]

		3-bromo-4,5-dihydroxybenzaldehyde; 2,4-dibromophenol; brominated derivatives of phlorotannins	Antioxidant Antimicrobials Anticancer	[29]
4.	*Chlorella sp***.	Caffeic acid Ferulic acid Gallic acid P-coumaric acid Chlorogenic acid	Antioxidant	[30; 31; 32]
*analytical evidence for bromphenols in *P. umbilicalis* lacking ** *Chlorella sp. does not produce phlorotannins and bromophenols*				

Studies performed on *Pseudomonas aeruginosa* (Fig. 6c) reveal that the bioproducts A5 and A8 have a moderate antimicrobial activity, with inhibition diameters of 16 mm and 10 mm, respectively. A local antimicrobial activity was also observed for bioproduct A6. Data obtained in the case of alcoholic extracts of Undaria pinnatifida agree by those obtained by Ferreira et al. [3], who reported 45% growth inhibition of P. aeruginosa using a bioproduct obtained via alcoholic extraction containing 1.5 mg/mL crude extract. Also, in the case of Chlorella sp., results obtained are in agree with those obtained by Shayma et al. [37] which have reported an inhibition diameter of 8 mm for an bioproducts derived from Chlorella sp., obtained from alcoholic media.

The results obtained from studies conducted on three types of Gram-negative bacteria (Fig. 7 a,b,c) showed the following:

- bioproducts obtained through aqueous extraction do not exhibit antimicrobial effects against the studied microorganisms;
- moderate antimicrobial effects were observed in the case of extracts A5 and A8, for *E. coli* (inhibition diameters of 15 mm) (Fig. 7a), *S. enterica* (Fig. 7b*)* (when are obtained the inhibition diameters of 15 mm and 13 mm, respectively), and S. marcescens (Fig. 7c) (when are obtained inhibition diameters of 18 mm and 14 mm, respectively);
- in the case of the bioproducts A6 and A7, obtained from brown algae, only local effects were obtained, in this case, the inhibition diameters ranged between (7 - 10) mm.

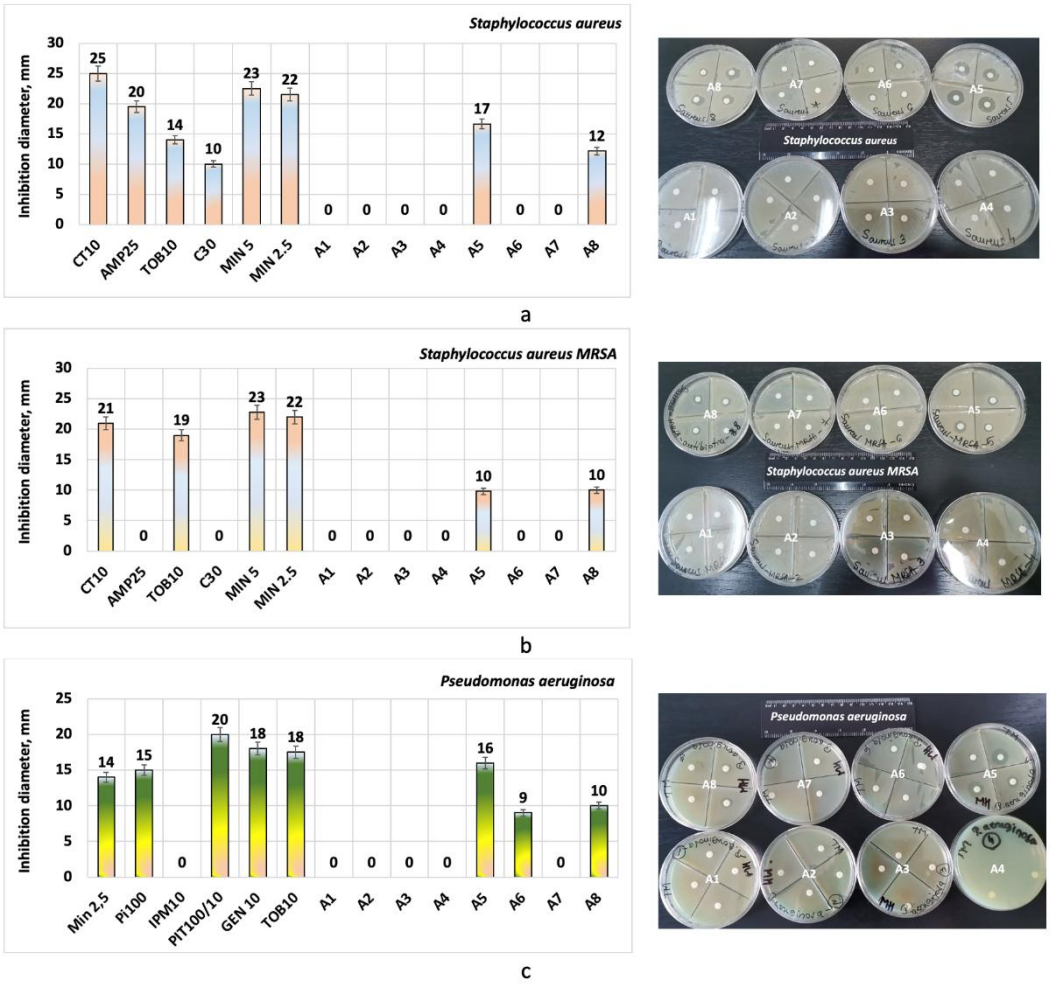

Fig. 6. Antimicrobial properties evaluation of algal bioproducts for three gram-positive bacteria: a) *Staphylococcus aureus*; b) *Staphylococcus aureus* MRSA; c) *Pseudomonas aeruginosa.* Source: Own studies

Similar results were obtained in studies conducted by Shaima et al., in the antimicrobial studies performed with a bioproduct containing 2 g/L crude alcoholic extracts from *Chlorella sp*. In this case, the average inhibition diameters obtained were 9 mm for *E. coli* and 8 mm for *S. marcescens* [37].

In the case of brown algae, Ferreira et al. [3] report that under the action of a bioproduct containing 1.5 mg/mL crude alcoholic extract, the growth of *E. coli* is inhibited by approximately 34%.

Freitas et al. [9] report no antimicrobial activity for *E. coli* at testing bioproducts obtained through alcoholic extraction from *Porphyra umbilicalis*. Karaman et al. report that when using diffusion discs containing 15 mg of a bioproduct obtained through alcoholic extraction from *Cystoseira barbata*, local

antimicrobial effects are observed for *Salmonella typhimurium* (inhibition diameter: 7 mm) and *E. coli* (inhibition diameter: 6.5 mm) [33]. Kosanić et al. reported a minimum inhibitory concentration of 5 mg/mL for crude alcoholic extracts obtained from *Cystoseira barbata* against *Escherichia coli* [35].

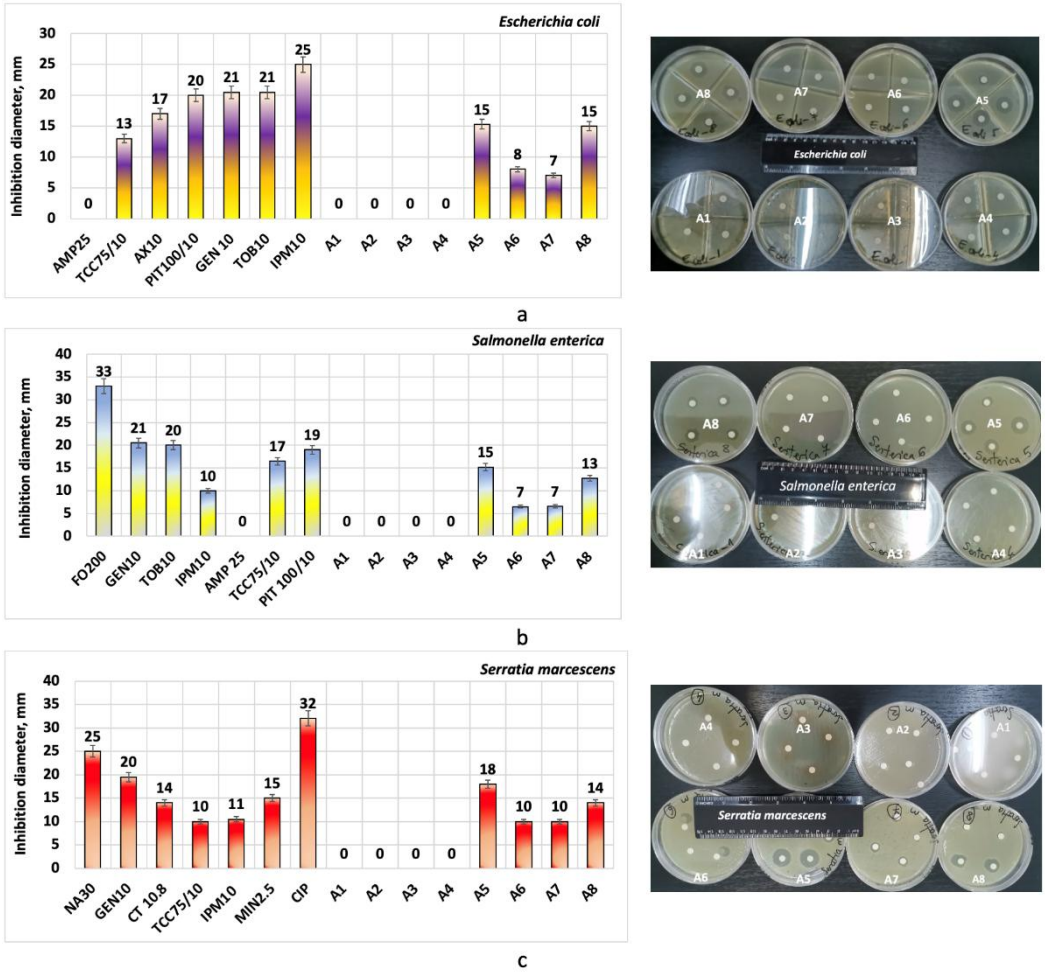

Fig. 7. Antimicrobial properties evaluation of algal bioproducts for three gram-negative bacteria: a) *Escherichia coli*; b) *Serratia marcescens*; c) *Salmonella enterica.* Source: Own studies

The results obtained in the case of the two pathogenic levures showed that the bioproducts derived from aqueous algae extracts (A1, A2, A3, A4) did not have an antimicrobial effect (Fig. 8 a,b). The bioproducts derived from the alcoholic extracts exhibit a moderate antimicrobial effect against *Candida albicans* (average inhibition diameter obtained ranged between (11–17) mm) and *Candida parapsilosis* (average inhibition diameter obtained ranged between (15–16) mm).

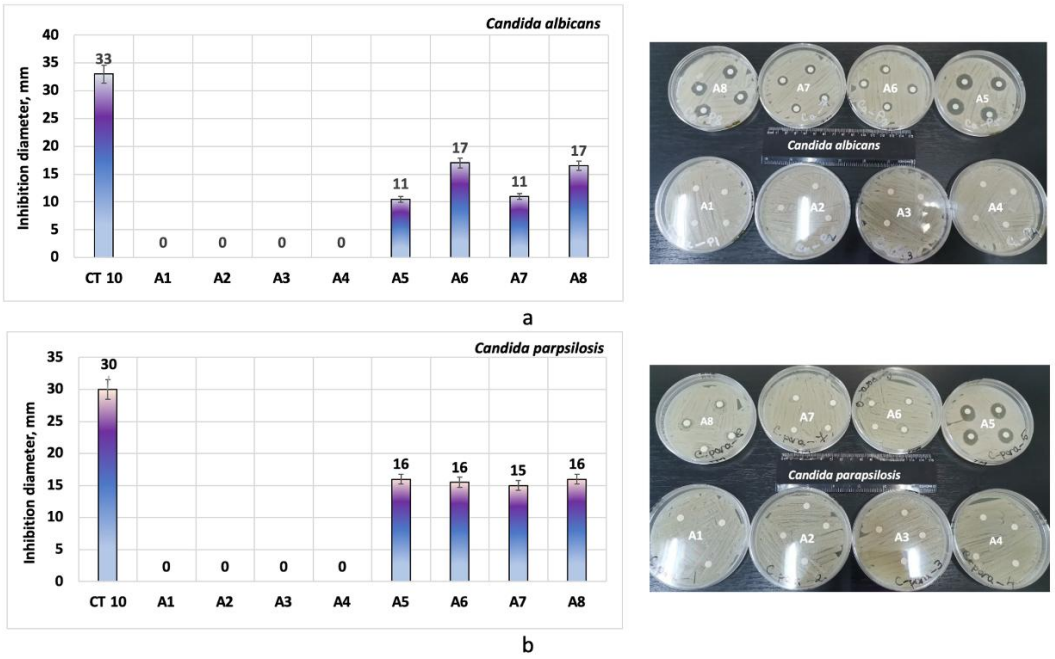

Fig. 8. Antimicrobial properties evaluation of algal bioproducts for two *Candida sp.* strains: a) *Candida albicans*; b) *Candida parapsilosis.* Source: Own studies

The best results were obtained for all bioproducts in the case of *Candida parapsilosis*, but the bioproducts A6 and A8 exhibited the best antimicrobial activity for both *Candida* strains. The anti-Candida activities of the bioproducts A6 and A7 may also be due to the bromophenols-based compounds found in both species of brown algae studied [25, 29] (Table 1).

These results are agreed with the studies conducted by Milović et al. [38] on bioproducts derived from *Cystoseira barbata*, which reported a MIC value for *Candida albicans* of 200 µg/mL. Kosanić et al. reported a MIC value of 2.5 mg/mL for Candida albicans for a crude extract obtained using Soxhlet extraction with acetone from *Cystoseira barbata* harvested from the Adriatic Sea [35]. Silva et al. documented the mechanism of action of phlorotannins against bacteria and fungi [13].

They reported that the antimicrobial activity is mainly due to the inhibition of the mitochondrial electron transport chain, disruption of the microbial membrane, and subsequent loss of microbial cell integrity. In the case of *Candida albicans*, phlorotannins also inhibit the formation of germ tubes (a transition state from budding to hyphal cells), reducing its virulence and implicit its capacity to adhere to epithelial cells [13]. The results obtained in this study confirm that the antimicrobial properties of marine plant biomaterials are comparable to those obtained from terrestrial plants [39-42].

Limitation of the studies performed. The antimicrobial tests performed in vitro using the Kirby Bauer methodology were conducted to preliminarily assess the effect of the eight (bio)materials on certain pathogenic microorganisms, in comparison with reference antibiotics reagents. The method used in this study provides only preliminary information because:

a)The tests are conducted *in vitro* in Petri dishes with solid culture media does not take into account physiological factors such as antibiotic molecule metabolism, the effects of the immune system, or the presence of biofilms;

b) The obtained results do not provide information about the minimum inhibitory concentration (MIC) [43, 44].

c) The method does not allow the testing of the effect of a mixture between the (bio)material tested and the antibiotic reagent, to evaluate synergy or antagonism resulting from the association between polyphenolic extracts with antimicrobial properties and certain antibiotics.

d) The technique cannot be applied to all types of microorganisms, as strict anaerobic bacteria require special culture conditions that are incompatible with the Kirby-Bauer method [45-46];

e) Intracellular bacteria (*Chlamydia, Rickettsia*), which grow only in living cells, cannot be tested using this method [45].

Future development the research performed. To evaluate with accuracy the antimicrobial activity of materials obtained from marine sources (algae), further studies (future research directions) are required, aiming to:

a) assessing the minimum inhibitory concentration of materials with antimicrobial effects.

b) evaluating any synergistic or antagonistic effects that may arise when these materials are combined with antibiotics specific to each tested microorganism.

Moreover, both types of materials (i.e. polysaccharides without antimicrobial activity, as well as materials enriched in polyphenols, with antimicrobial properties) can be tested to assess other biological properties, such as antitumor or antioxidant effects.

Research into the synergy or antagonism between these materials and other cytostatic agents could be an interesting area of study, particularly as previous research has highlighted positive results obtained in vitro, as well as in preclinical and clinical studies, through the combination of natural (bio)materials with or without antitumor drugs [47-50].

These studies are significant in the context of the circular bioeconomy, as they use sustainable and renewable materials to develop new (bio)materials containing biomolecules with antibiotic effects or new biosorbents for heavy metal removal from industrial wastewater [49].

4. Conclusion

Crude bioproducts obtained by Soxhlet extraction in alcoholic media from *Porphyra umbilicalis* and *Chlorella sp*. exhibit moderate or local antimicrobial properties against *Staphylococcus aureus*, *Staphylococcus aureus* MRSA, and *Pseudomonas aeruginosa*. In the latter case, local activities were observed only for the extract derived from *Undaria pinnatifida*.

For Gram-negative bacteria, bioproducts derived from alcoholic extracts exhibit local or moderate antimicrobial properties against microorganisms such as *Escherichia coli*, *Serratia marcescens*, and *Salmonella enterica*. Here, the best activities were found in the case of the bioproducts derived from *Porphyra umbilicalis* and *Cystoseira barbata*.

Regarding the behaviour of the studied bioproducts against two *Candida* species, results showed that only bioproducts obtained from alcoholic media exhibit anti-*Candida* properties. The best anti-*Candida* activities were found for the bioproducts derived from *Undaria pinnatifida* and *Cystoseira barbata*.

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