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A Prospect on the Antimicrobial Activity of Algae Extract : The Fucales Order Case. †

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Abstract: Over the years, foodborne pathogens have caused countless health problems and massive financial losses. Therefore, an essential goal for the food industry is to prevent food contamination and the related foodborne illnesses as microbial contamination of food items during their acquiring and distribution processes is still a hygienic issue. Moreover, there is an important movement leading to the pursuit of more natural and safe food supplies and ingredients with a special emphasis in the vegan and vegetarian community, as a result, there is a resurgence in demand for natural and eco-friendly products as a replacement for synthetic ingredients.In this context, and due to their active substances, macroalgae stand out as they are known for possessing antibacterial qualities among other abilities. Because of this, the current study updates our understanding of microbial pollutants in the food industry and compile the last updates on the scientific reports on antimicrobial activity of the edible brown algae species with special attention to the algae *Bifurcaria bifurcata*, *Fucus spiralis* and *Ascophyllum nodosum*. These species which belong to the Phaeophyceae class and order Fucales are reported as rich in active compounds and are still an undervalued resource. So, the ability of algal extracts to stop the growth of various significant food pathogens was reviewed while considering their advantageous effects on food safety and quality issues.

Keywords: Fucales; antimicrobial activity; foodborne pathogens

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1. Introduction

Rich in biodiversity, the oceans have gained global importance and are increasingly under scrutiny as a source of natural products. Among the many organisms living in marine habitats, macroalgae have attracted much interest due to their diversity and potent bioactive metabolites [1]. Members of the Fucales family, a particular group of brown macroalgae, are known for their ecological importance, metabolite composition, and potent bioactive properties.

These metabolites, ranging from polysaccharides[2] to phenolic compounds [3] and terpenoids [4], not only contribute to the ecological interactions of the algae but also possess promising bioactive properties. This work focuses on exploring the antimicrobial potential of extracts from macroalgae belonging to the Fucales family, namely *Bifurcaria bifurcata*, *Fucus spiralis*, and *Ascophyllum nodosum*. Figure1 presents photographs of this species in detail and in their natural environment. The study aims to elucidate the spectrum

of antimicrobial activity of these extracts against a range of significant microorganisms highlighting food related pathogens.

Figure1- Macroalgae discussed in this work: detailed outlook and in their natural environment.

2. Discussion

With the aim of evaluating the latest published developments in the field of antimicrobial capacity of algal extracts, available databases were searched using the name of the alga and antimicrobial activity as keywords. A summary of the main results published in the last five years is presented in Table 1.

Several important foodborne microorganisms can cause various diseases when ingested through contaminated food. Some of the most important of these are*: Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*[5]. Macroalgal extracts could play a role as inhibitors of these pathogens[6,7] . For example, the pathogenic effect of toxins produced by *E. coli* is one of the most important causes of foodborne illness worldwide[7,8]. Moreover, they are studies also supporting the inclusion of alga extracts as food preserves [9,10].

The antimicrobial activity of *B. bifurcaria* extracts obtained by maceration with solvents of different polarity has been studied previously. The results highlighted the potential of *B. bifurcaria* as an effective antimicrobial agent, as all extracts were active against five of the six microorganisms tested [11]. In another work, the algal extract (hexane–isopropanol–water (10:80:10)) was tested against *Bacillus cereus*, *Bacillus subtilis*, *Geobacillus*

stearothermophilus, *L. monocytogenes*, *S.aureus*, and *Staphylococcus haemolyticus*. The results showed antimicrobial activity against all microorganisms, with seasonal variation in activity and minimum inhibitory concentrations ranging from 0.3 mg/mL (*G. stearothermophilus*) to 19.9 mg/mL (*S. aureus*) [12]. In addition, the water extracts of *B. bifurcaria* were found to have potent antifungal activity against *Penicillium digitatum*, *Penicillium expansum*, and *Penicillium italicum* [13].

Table 1. Selected studies on the antimicrobial activity of namely *Bifurcaria bifurcata*, *Fucus spiralis*, and *Ascophyllum nodosum*.

Extraction technique	Microorganism tested	Major results	Ref.
	Bifurcaria bifurcata		
Sequential extraction (RT); (Hx, MeOH,Wt) 1:20 (m/v):	*Epidermophyton floccosum, Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes var. interdigitale; Thrichophyton rubrum, Trichophyton verrucosum.*	MeOH extracts demonstrated antifungal capacity against human dermatophyte fungi. The antifungal activity seems to be seasonally /geographically influenced	[14]
Maceration 50ºC, 24h EtOH, AcO, EtAc,Chl and Hex	*Staphylococcus aureus Staphylococcus epidermidis, Bacillus cereus Pseudomonas aeruginosa Salmonella enteritidis Escherichia coli*	Strong inhibition activity, all extracts were active against all microorganisms except *E. coli*	[11]
Maceration RT 60 min Hx–IPr-W (10:80:10)	*Bacillus cereus; Bacillus subtilis; Geobacillus stearothermophilus; Listeria monocytogenes; Staphylococcus aureus; Staphylococcus haemolyticus;*	MICs values between 0.9 mg/mL (*B.cereus*) and >19.9 (*L. monocytogenes)* spatial and seasonal variations. Inconsistencies between disc diffusion and broth dilution methods.	[12]
Maceration RT , 4 days MeOH	*Penicillium digitatum, Botrytis cinerea*	Active against both microorganisms at the 4 harvest seasons tested	[15]
Maceration 48h , RT, + 30 min. ultrasonication	*Penicillium digitatum, Penicillium expansum Penicillium italicum*	Strong antifungal activity, effective in reducing the mycelial growth.	[13]
maceration RT 48h methanol (90%)	*E. coli, Staphylococcus aureus Bacillo subtilis P. aeruginosa*	MICs from 0.11 to 1.87 mg/mL	[13]
Sequential extraction MeOH; DCM/MeOH (50:50), DCM	*Escherichia coli Proteus mirabilis Staphylococcus aureus* CECT 976 *Staphylococcus aureus* ATCC 25923	MIC of 0.02 µg/ml against P. mirabilis, 0.3 µg/ml against S. aureus CECT 976 and 1.8 µg/ml against the S.aureus ATCC 25923.	[16]
	Fucus Spiralis		
Maceration RT , 4 days MeOH	*Penicillium digitatum Botrytis cinerea*	Algae harvest in summer was active against both fungal species	[15]
Maceration RT: overnigth DCM:MeOH (1:1) extract of Fucus spiralis: PE EtAc n-But	*Staphylococcus aureus, Bacillus subtilis Bacillus cereus, Escherichia coli, Proteus mirabilis,Mucor mucedo, Trichophyton mentagrophytes, Aspergilus*	The crude extract and fractions were active against all tested microbes, the best result was obtained with the lipidic fraction.	[17]

	niger. Candida albicans, Penicillium italicum		
Maceration 50ºC, 24h EtOH, AcO, EtAc,Chl and Hex, alga 0.03 g per mLof solvent	*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Pseudomonas aeruginosa. Salmonella enteritidis Escherichia coli*	Acetonic extract was de most active	[11]
Sequential extraction cHx; EtAc , EtOH/ Maceration EtOH,W/Shoxleth EtOH (frations W, Dieth, EtAc)	*Staphylococcus epidermidis, Cutibacterium acnes, Malassezia furfur*	The concentration used (1 mg/mL) is not effective against the studied microorganisms.	[18]
Maceration AcO:W(7:3) and purification to obtain phlorotannins	*Epidermophyton floccosum, Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum canis and Microsporum gypseum*	MIC values raking from 7.8 to 31.3 mg/mL against skin and nails isolated fungus	[19]
Sequential extraction MeOH, DCM/MeOH(50:50)/ DCM	*Escherichia coli, Proteus mirabilis, Staphylococcus aureus CECT 976, Staphylococcus aureus, ATCC 25923*	MIC of 3.6 µg/ml against P. mirabilis 2.7 µg/ml against S. aureus CECT 976 and 10.65 µg/ml against the S. aureus ATCC 25923.	[16]
	Ascophyllum nodosum		
Maceration RT 30min MeOH:W (1:1)	*Escherichia coli*	*Ascophyllum nodosum* revealed antioxidant and antimicrobial capacity	[20]
Shoxleth ACO 6h:	*Escherichia coli*	Antimicrobial effect against E.coli in the first 6hours	[21]
Maceration AcO: W (7:3) 3h RT and purification solid-phase extraction (SPE)	*Escherichia coli, O157:H7 Salmonella agona Streptococcus. suis*	Mics of raking from 0.78 to 1.56 mg/mL	[6]
Maceration 50ºC, 24h EtOH, AcO, EtAc,Chl and Hex,	*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Pseudomonas aeruginosa, Salmonella enteritidis, Escherichia coli*	The ethanolic extract was de most active	[11]

AcO Acetone, nBut n-butanol, Chl chloroform, cHx cyclohexane, DCM dichloromethane Dieth diethyl ether, EtOH Ethanol, EtAc ethyl acetate, HX -hexane; IPr isopropanol MeOH methanol, PE petroleum ether W water, RT room temperature

This antifungal activity was also confirmed by studies conducted with methanolic extracts against *P digitatum* and *Botrytis cinerea* [15]. In another study [14], the activity of methanolic extract was used in interrupting the growth of dermatophytic fungi that. The authors concluded that the algal alga has an important inhibitory activity for the action on *Epidermophyton floccosum* and is one of the most effective algae in the published literature. In addition, the preservative effect of *B. bifurcata* extracts on the quality of chilled hake quality shake was tested. and the results highlight the antimicrobial effect of aqueous and ethanolic *B. bifurcata* extracts in the icing media and demonstrate the potential of macroalgae bioactive compounds to preserve food quality [9].

Similarly, extracts of *Fucus spiralis* have been tested as antimicrobial and antifungal agents. A study on the antifungal activity of the methanolic extract of *F. Spiralis* against *P.*

digitatum and *Botrytis cinerea* [15] showed a moderate effect, but pointed out the seasonal variation of this property, since only the extracts from the alga harvested in summer were active. Inhibition of various Gram-positive and Gram-negative microorganisms is described by N. Grozdanic et al. The most active antibacterial effect was achieved by the n-butanol fraction with Mics values ranging from 0.04 mg/mL for *B. cereus* and *B. subtilis* to 0.14 mg/mL for *P. mirabilis* and *E.coli* . In the same study, the effect on the fungi *Mucor mucedo*, T*richophyton mentagrophytes*, *Aspergilus niger*, *C. albicans*, and *P. italicum* was also investigated. In both cases (antimicrobial and antifungal activity), the lipid fraction was the most active [17]. This results agree with previous work stating the inhibition of several fungi by phlorotannis from *F.spirailis* [19]. The activity of *F spiralis* extracts prepared by heat-assisted extraction with solvents of different polarity, as solvent properties are an important factor for antimicrobial activity [22]. The results showed that the most active extract was obtained when acetone was used as solvent, which was active against *S. aureus*, *B.cereus*, and *Salmonella enteritidis*. [11]. The antimicrobial activity of *F. spiralis* extracts was also confirmed by results obtained with dichloromethane/methanol as extracting solvent, which showed minimal inhibitory concentrations ranging from 2.7 ug/mL *(S. aureus*) to 1875 ug/mL *(E. coli).* Among other possibilities, *F. spiralis* extracts can be used for dermo-cosmetic applications, as they could contribute to the maintenance of a healthy skin microbiota [18].

The antimicrobial capacity of *Ascophyllum nodosum* was studied in vitro against *E. coli* serotype O138. The results show a dose-dependent relationship between the inhibitory activity and the concentration of the extract [20], these results agree with those of Dell'Anno et al, they found an antibacterial action against *E. coli* O138 evidenced by a decrease in bacteria growth after 3 hours attained by 0.12% *Ascophyllum nodosum* extract concentration, demonstrating a dose-dependent inhibitory effect[21].

The antimicrobial efficacy of an acetone-water mixture (7:3, v/v, 2 mL) *A. nodosum* extract purified phlorotannin's against *E.coli*. O138, *Salmonella agona*, and *Streptococcus suis* has been examined before and showed a range of MICs for the different pathogens between 1.56 and 0.78 mg/mL. The authors also examined cell membrane permeability and intracellular adenosine triphosphate (ATP) to establish the inhibitory mechanism. They conclude that phlorotannin extracts dramatically lowered the intracellular ATP levels of all three microorganisms. Importantly, when subjected to the same or higher dosages that have been proven to inhibit bacterial growth (up to 25 mg/ml), the phlorotannin extracts exhibited no negative effects on pig intestinal cells, suggesting that they could be used as an alternative and supplement to antibiotics and zinc in animal diets [6].

In summary, we find that the three species of algae studied in this mini-review have significant antimicrobial capacity, although it is worth pointing out that several factors affect their antimicrobial performance, from the extraction technique used to variations in maturation and provenience of the algal material. However, their applicability, e.g., as an aid in food preservation, is high and is an interesting topic of future research in applied technology.

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References

1. Hakim, M.M.; Patel, I.C. A Review on Phytoconstituents of Marine Brown Algae. *Futur. J. Pharm. Sci.* **2020**, *6*, 129, doi:10.1186/s43094-020-00147-6.
2. Li, Y.; Zheng, Y.; Zhang, Y.; Yang, Y.; Wang, P.; Imre, B.; Wong, A.C.Y.; Hsieh, Y.S.Y.; Wang, D. Brown Algae Carbohydrates: Structures, Pharmaceutical Properties, and Research Challenges. *Mar. Drugs* **2021**, *19*, 620, doi:10.3390/md19110620.
3. Catarino, M.D.; Pires, S.M.G.; Silva, S.; Costa, F.; Braga, S.S.; Pinto, D.C.G.A.; Silva, A.M.S.; Cardoso, S.M. Overview of Phlorotannins' Constituents in Fucales. *Mar. Drugs* **2022**, *20*, 754, doi:10.3390/md20120754.
4. Pais, A.C.S.; Saraiva, J.A.; Rocha, S.M.; Silvestre, A.J.D.; Santos, S.A.O. Current Research on the Bioprospection of Linear Diterpenes from *Bifurcaria Bifurcata:* From Extraction Methodologies to Possible Applications. *Mar. Drugs* **2019**, *17*, 556, doi:10.3390/md17100556.
5. Kumaran, S.; Deivasigamani, B.; Alagappan, K.; Sakthivel, M.; Karthikeyan, R. Antibiotic Resistant Esherichia Coli Strains from Seafood and Its Susceptibility to Seaweed Extracts. *Asian Pac. J. Trop. Med.* **2010**, *3*, 977–981, doi:10.1016/S1995-7645(11)60013-8.
6. Ford, L.; Stratakos, A.C.; Theodoridou, K.; Dick, J.T.A.A.; Sheldrake, G.N.; Linton, M.; Corcionivoschi, N.; Walsh, P.J. Polyphenols from Brown Seaweeds as a Potential Antimicrobial Agent in Animal Feeds. *ACS Omega* **2020**, *5*, 9093–9103, doi:10.1021/acsomega.9b03687.
7. Silva, A.; Silva, S.A.; Lourenço-Lopes, C.; Jimenez-Lopez, C.; Carpena, M.; Gullón, P.; Fraga-Corral, M.; Domingues, V.F.; Barroso, M.F.; Simal-Gandara, J.; et al. Antibacterial Use of Macroalgae Compounds against Foodborne Pathogens. *Antibiotics* **2020**, *9*, 712, doi:10.3390/antibiotics9100712.
8. Smith, J.; Fratamico, P. Escherichia Coli as Other Enterobacteriaceae: Food Poisoning and Health Effects. In *The Encyclopedia of Food*; Caballero, B., Finglas, P., Toldra, F., Eds.; Oxford Acad., 2016; pp. 539-544.
9. Miranda, J.M.; Zhang, B.; Barros-Velázquez, J.; Aubourg, S.P. Preservative Effect of Aqueous and Ethanolic Extracts of the Macroalga *Bifurcaria Bifurcata* on the Quality of Chilled Hake (Merluccius Merluccius). *Molecules* **2021**, *26*, 3774, doi:10.3390/molecules26123774.
10. Pisoschi, A.M.; Pop, A.; Georgescu, C.; Turcuş, V.; Olah, N.K.; Mathe, E. An Overview of Natural Antimicrobials Role in Food. *Eur. J. Med. Chem.* **2018**, *143*, 922–935, doi:10.1016/j.ejmech.2017.11.095.
11. Silva, A.; Rodrigues, C.; Garcia-Oliveira, P.; Lourenço-Lopes, C.; Silva, S.A.; Garcia-Perez, P.; Carvalho, A.P.; Domingues, V.F.; Barroso, M.F.; Delerue-Matos, C.; et al. Screening for Bioactive Properties on Brown Algae from the Northwest Iberian Peninsula. *Foods* **2021**, *10*, 1–15, doi:10.3390/foods10081915.
12. Rubiño, S.; Peteiro, C.; Aymerich, T.; Hortós, M. Brown Macroalgae (Phaeophyceae): A Valuable Reservoir of Antimicrobial Compounds on Northern Coast of Spain. *Mar. Drugs* **2022**, *20*, 775, doi:10.3390/md20120775.
13. Fayzi, L.; Askarne, L.; Boufous, E.H.; Cherifi, O.; Cherifi, K. Antioxidant and Antifungal Activity of Some Moroccan Seaweeds Against Three Postharvest Fungal Pathogens. *Asian J. Plant Sci.* **2022**, *21*, 328–338, doi:10.3923/ajps.2022.328.338.
14. Carvalho, G.L.; Silva, R.; Gonçalves, J.M.; Batista, T.M.; Pereira, L. Extracts of the Seaweed *Bifurcaria Bifurcata* Display Antifungal Activity against Human Dermatophyte Fungi. *J. Oceanol. Limnol.* **2019**, *37*, 848–854, doi:10.1007/s00343-019-8118-9.
15. N. Bahammou; Raja, R.; Carvalho, I.S.; Cherifi, K.; Bouamama, H.; Cherifi, O. Assessment of the Antifungal and Antioxidant Activities of the Seaweeds Collected from the Coast of Atlantic Ocean, Morocco. *Moroccan J. Chem.* **2021**, *9*, 639–648, doi:10.48317/IMIST.PRSM/morjchem-v9i3.25910.
16. Benhniya, B.; Lakhdar, F.; Rezzoum, N.; Etahiri, S. GC/MS Analysis and Antibacterial Potential of Macroalgae Extracts Harvested on Moroccan Atlantic Coast. *Egypt. J. Chem.* **2022**, 0–0, doi:10.21608/ejchem.2022.117053.5301.
17. Grozdanic, N.; Djuricic, I.; Kosanic, M.; Zdunic, G.; Savikin; Katarina; Etahiri; Samira; Assobhei; Omar; et al. *Fucus Spiralis* Extract and Fractions: Anticancer and Pharma- Cological Potentials. *J. Boun* **2020**, *25*.
18. Freitas, R.; Martins, A.; Silva, J.; Alves, C.; Pinteus, S.; Alves, J.; Teodoro, F.; Ribeiro, H.M.; Gonçalves, L.; Petrovski, Ž.; et al. Highlighting the Biological Potential of the Brown Seaweed *Fucus Spiralis* for Skin Applications. *Antioxidants* **2020**, *9*, 611, doi:10.3390/antiox9070611.
19. Lopes, G.; Pinto, E.; Andrade, P.B.; Valentão, P. Antifungal Activity of Phlorotannins against Dermatophytes and Yeasts: Approaches to the Mechanism of Action and Influence on Candida Albicans Virulence Factor. *PLoS One* **2013**, *8*, e72203, doi:10.1371/journal.pone.0072203.

20. Frazzini, S.; Scaglia, E.; Dell'Anno, M.; Reggi, S.; Panseri, S.; Giromini, C.; Lanzoni, D.; Sgoifo Rossi, C.A.; Rossi, L. Antioxidant and Antimicrobial Activity of Algal and Cyanobacterial Extracts: An In Vitro Study. *Antioxidants* **2022**, *11*, 992, doi:10.3390/antiox11050992.
21. Dell'Anno, M.; Sotira, S.; Rebucci, R.; Reggi, S.; Castiglioni, B.; Rossi, L. In Vitro Evaluation of Antimicrobial and Antioxidant Activities of Algal Extracts. *Ital. J. Anim. Sci.* **2020**, *19*, 103–113, doi:10.1080/1828051X.2019.1703563.
22. Vicente, T.F.L.; Félix, C.; Félix, R.; Valentão, P.; Lemos, M.F.L. Seaweed as a Natural Source against Phytopathogenic Bacteria. *Mar. Drugs* **2022**, *21*, 23, doi:10.3390/md21010023.