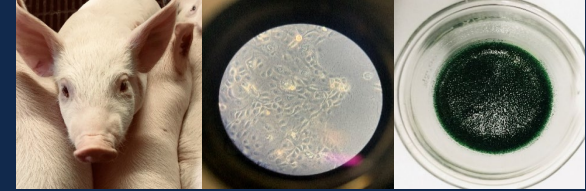




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Ministry of Science and Higher Education
Republic of Poland



Algal-based extracts and their mixes: ability to perform antimicrobial and antioxidant activities

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DIPARTIMENTO DI MEDICINA
VETERINARIA E SCIENZE ANIMALI





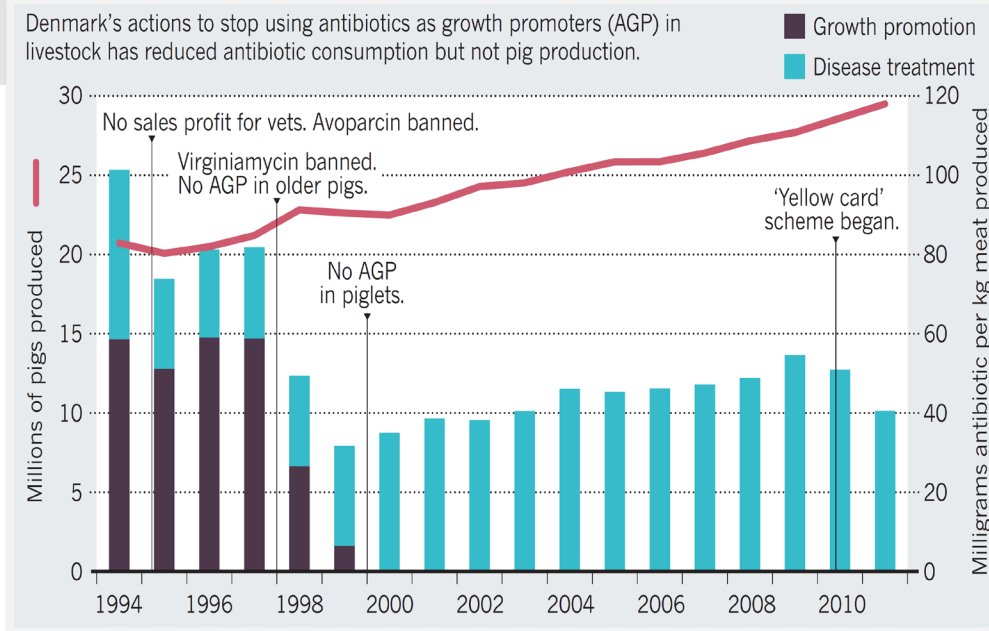
ANTIBIOTICS AS GROWTH PROMOTING AGENTS AND ANTIMICROBIAL RESISTANCE AS GLOBAL THREAT



Reducing antibiotic use in animal production decreased the prevalence of antibiotic-resistant bacteria in animals about **15%** and multidrug-resistant bacteria about **24-32%**.



www.efsa.europa.eu/it/interactivepages/antimicrobialresistance



* Tang et al., WHO (2017).

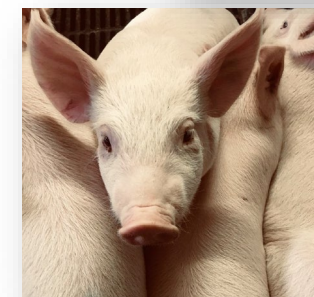


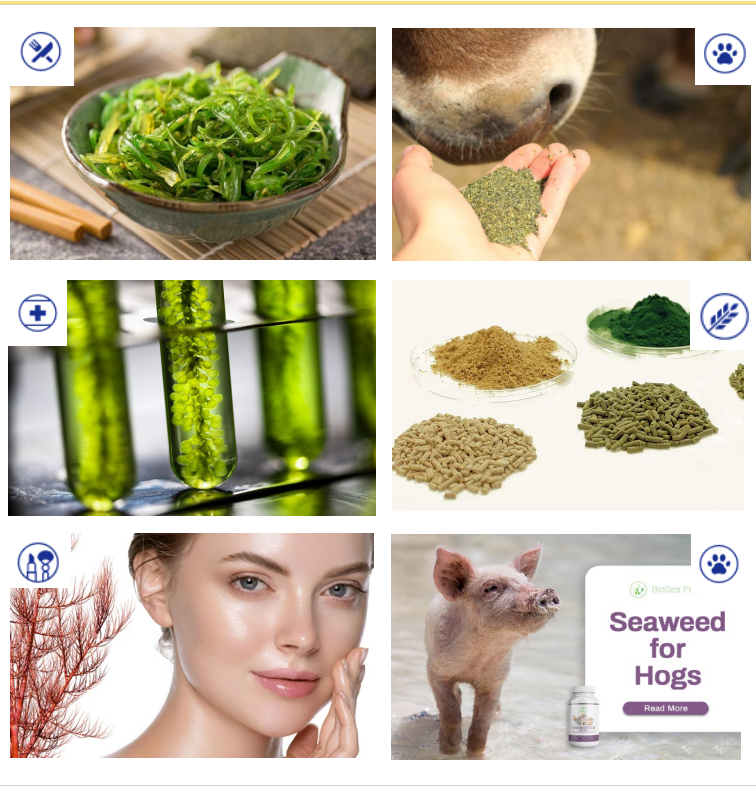
Fig. 1. Consumption of antimicrobials for use as AGPs (red bars) or for therapeutic administration (blue bars) in Danish swine farms.

* Aarestrup et al. (2012).

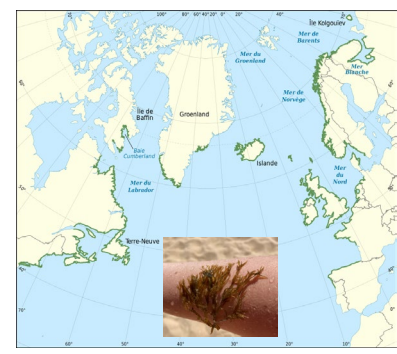
INTRODUCTION



SEAWEEDS AS ANTIBIOTIC ALTERNATIVES



Ascophyllum nodosum

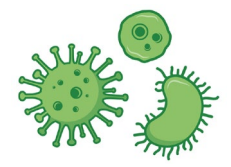


ALGAE AS ALTERNATIVES

1 ANTIBACTERIAL PROPERTIES



AN, PP and ANPP EXPERIMENT I



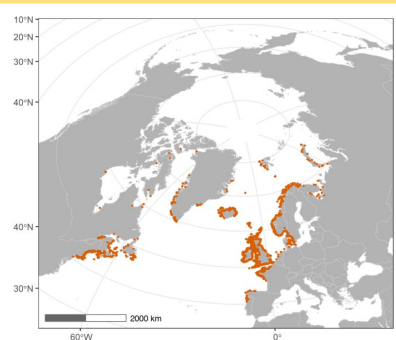
2 ANTIOXIDANT PROPERTIES



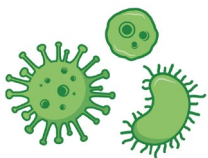
AN, PP and ANPP EXPERIMENT II



Palmaria palmata



INTRODUCTION



SEAWEEDS – HOW THEY INFLUENCE ON BACTERIA GROWTH

The aim of the study was to test the selected algae extracts and their mixes and **their inhibitory activity against ETEC and VTEC *Escherichia coli* strains (F4+ and F18+)** through liquid culture of *Escherichia coli*.



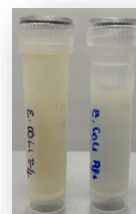
AN, PP and ANPP



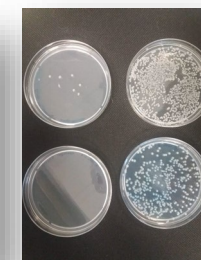
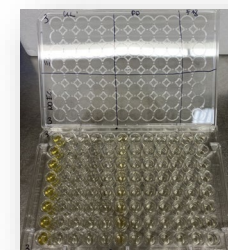
**ALGAL
EXTRACTS**



Escherichia coli



**ETEC F4+
VTEC F18+**



**Influence on growth
inhibition**

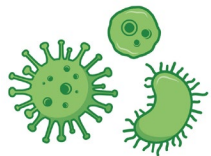


EXPERIMENT I

AIM OF THE STUDY



ANTIBACTERIAL PROPERTIES

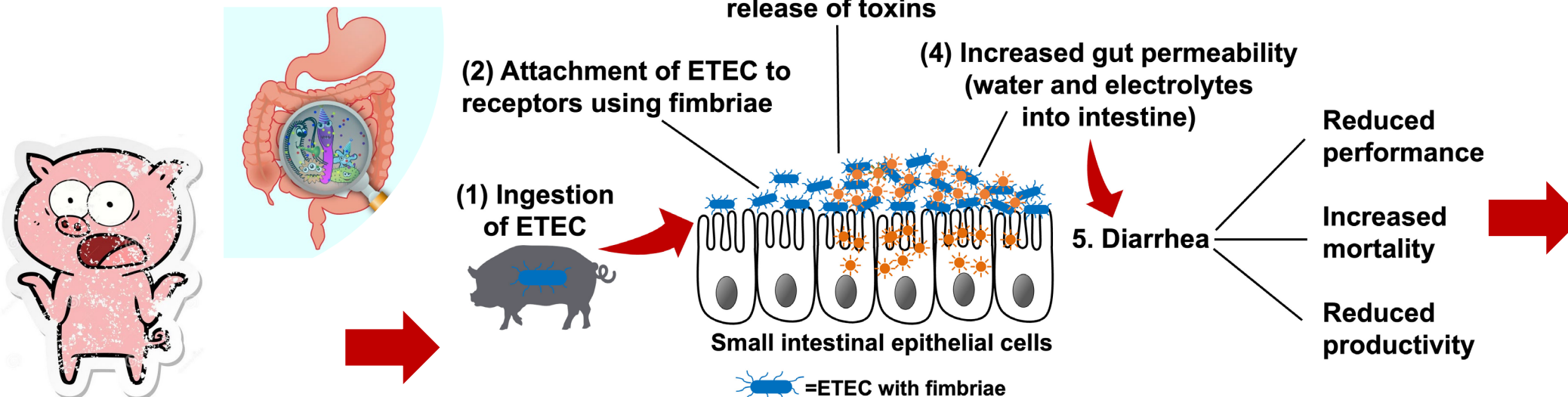


SEAWEEDS – HOW THEY INFLUENCE ON BACTERIA GROWTH

WEANING STRESS

BACTERIAL INFECTIONS

ANTIBIOTHERAPY

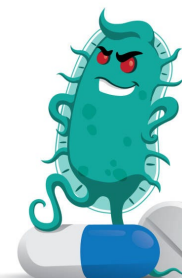


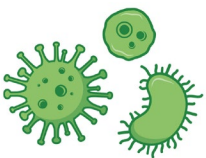
EXPERIMENT I

INTRODUCTION

Escherichia coli F4+ F18+

ANTIBACTERIAL PROPERTIES





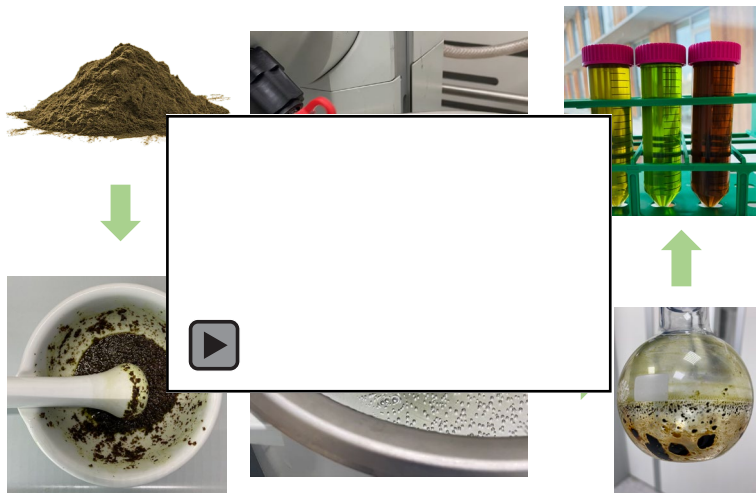
SEAWEEDS – HOW THEY INFLUENCE ON BACTERIA GROWTH

A liquid culture-based *Escherichia coli* F4+ and F18+ was supplemented with seaweeds extracts and their mixes with an experimental design:

2 (with or without *E. coli*) × 2 (F4+ and F18+) × 5 doses (0, 1.44, 2.87, 5.75, 11.50, and 23.00 mg/mL of seaweed extracts).

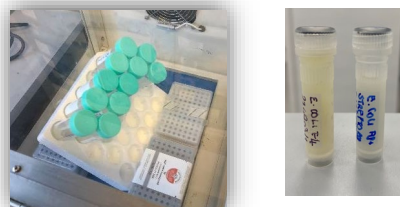
1

ALGAE POWDERS EXTRACTION PROCESS



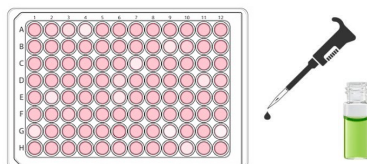
2

12h grown at 37°C with shaking
(150 × rpm) in lysogeny broth (LB)



3

Inoculation of different EC in 96-
wells with doses of seaweeds

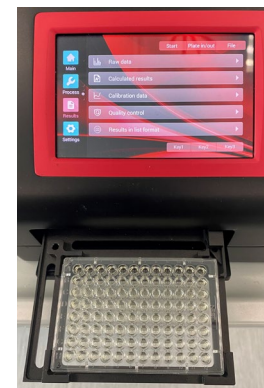


4

ABSORBANCE MEASUREMENT

$$\text{Inhibition rate (\%)} = 100 * \left(\frac{OD_{CTRL^-} - OD_{sample}}{OD_{CTRL^-} - OD_{blank}} \right)$$

In 60-mins intervals



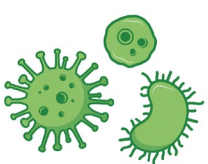
EXPERIMENT I

METHODOLOGY

ANTIBACTERIAL PROPERTIES

Data were analyzed (SAS 9.4)

sas



SEAWEEDS – HOW THEY INFLUENCE ON BACTERIA GROWTH

- AN species dose-dependently **significantly inhibited** ($p < 0.05$) the growth of ETEC F4+ *in vitro* in all-time points.
- PP **significantly inhibited** the growth in the highest dose in both T1, T2.
- ANPP did not inhibit ETEC F4+ growth.

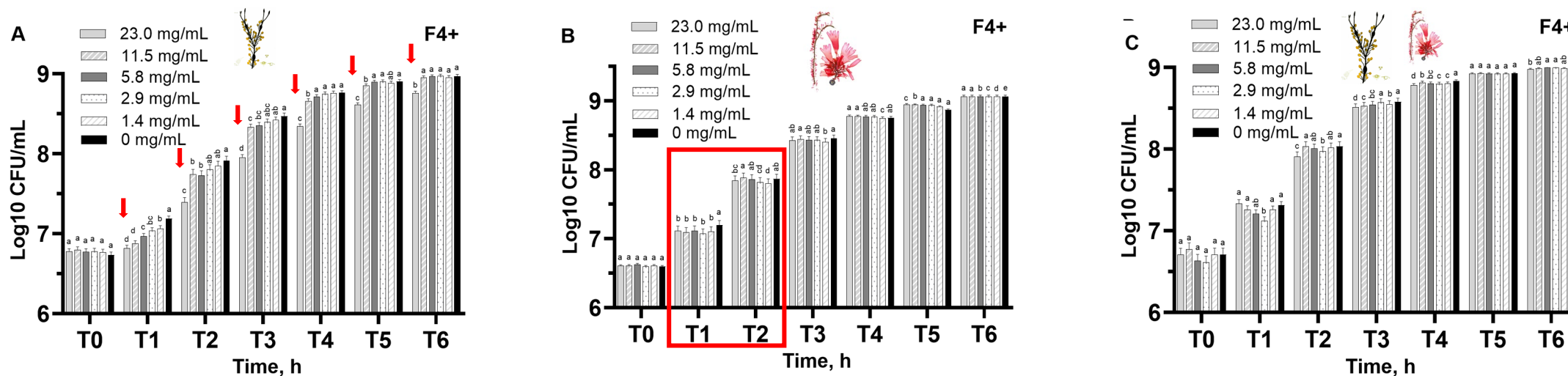
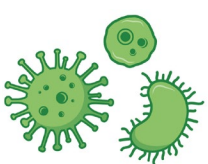


Fig. 2. The impact of different concentrations (0, 1.44, 2.87, 5.75, 11.50, and 23.00 mg/mL) of AN (A), PP (B) and ANPP (C) on Escherichia coli F4+ growth in 60-min time interval points (T0, T1, T2, T3, T4, T5, T6). Data are expressed as log10 CFU/mL LSMEAN $\pm$ SEM ($n = 3$). Different superscript letters express significant differences at $p < 0.05$ among different concentrations within the same time point.

RESULTS



ANTIBACTERIAL PROPERTIES



SEAWEEDS – HOW THEY INFLUENCE ON BACTERIA GROWTH

- AN species also dose-dependently **significantly inhibited** ($p < 0.05$) the growth of VTEC F18+ *in vitro*, with the strongest response of highest dose.
- PP **significantly inhibited** the growth in T2, T3 and T4.
- ANPP **also inhibited** the VTEC F18+ growth starting from T2.

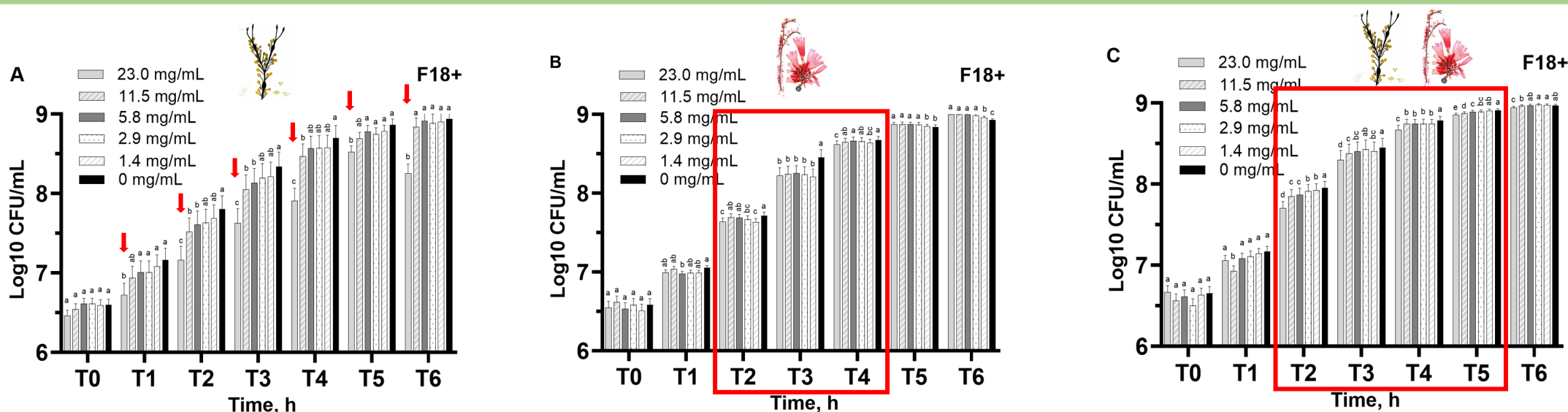
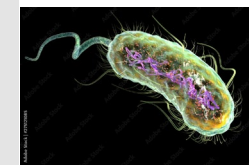


Fig. 3. The impact of different concentrations (0, 1.44, 2.87, 5.75, 11.50, and 23.00 mg/mL) of AN (A), PP (B) and ANPP (C) on Escherichia coli F18+ growth in 60-min time interval points (T0, T1, T2, T3, T4, T5, T6). Data are expressed as log₁₀ CFU/mL LSMEAN ± SEM (n = 3). Different superscript letters express significant differences at $p < 0.05$ among different concentrations within the same time point.

EXPERIMENT I

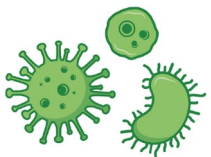
RESULTS



ANTIBACTERIAL PROPERTIES



EAAP
European Association
of Animal Pathologists



SEAWEEDS – HOW THEY INFLUENCE ON BACTERIA GROWTH

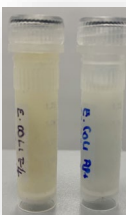
In this study, the brown macroalgae *Ascophyllum nodosum* extracts were the most effective in terms of inhibition of both strains of *Escherichia coli*, thus may possess strong antibacterial activity.



AN, PP and ANPP



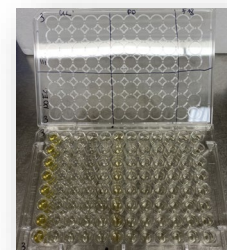
**ALGAL
EXTRACTS**



Escherichia coli



**ETEC F4+
VTEC F18+**



Inhibition of growth

CONCLUSION

ANTIBACTERIAL PROPERTIES

EXPERIMENT I

SEAWEEDS – THEIR ANTIOXODANT PROPERTIES

The aim of this study was to **test the antioxidant activity** of seaweeds extracts through two chemical-based assays.

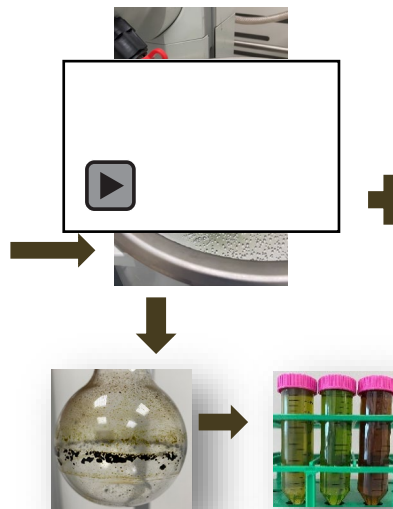
ANTIOXIDANT

ALGAL EXTRACTS

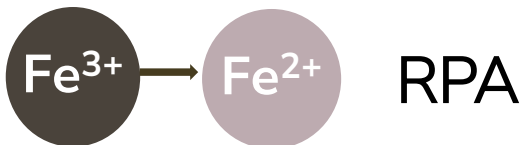


ALGAL EXTRACTS

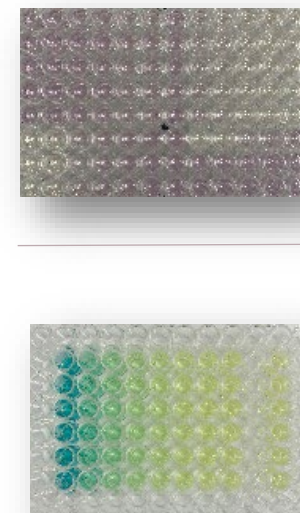
AN, PP and mixes
(AN50%-PP50%)



DPPH



RPA



EXPERIMENT II

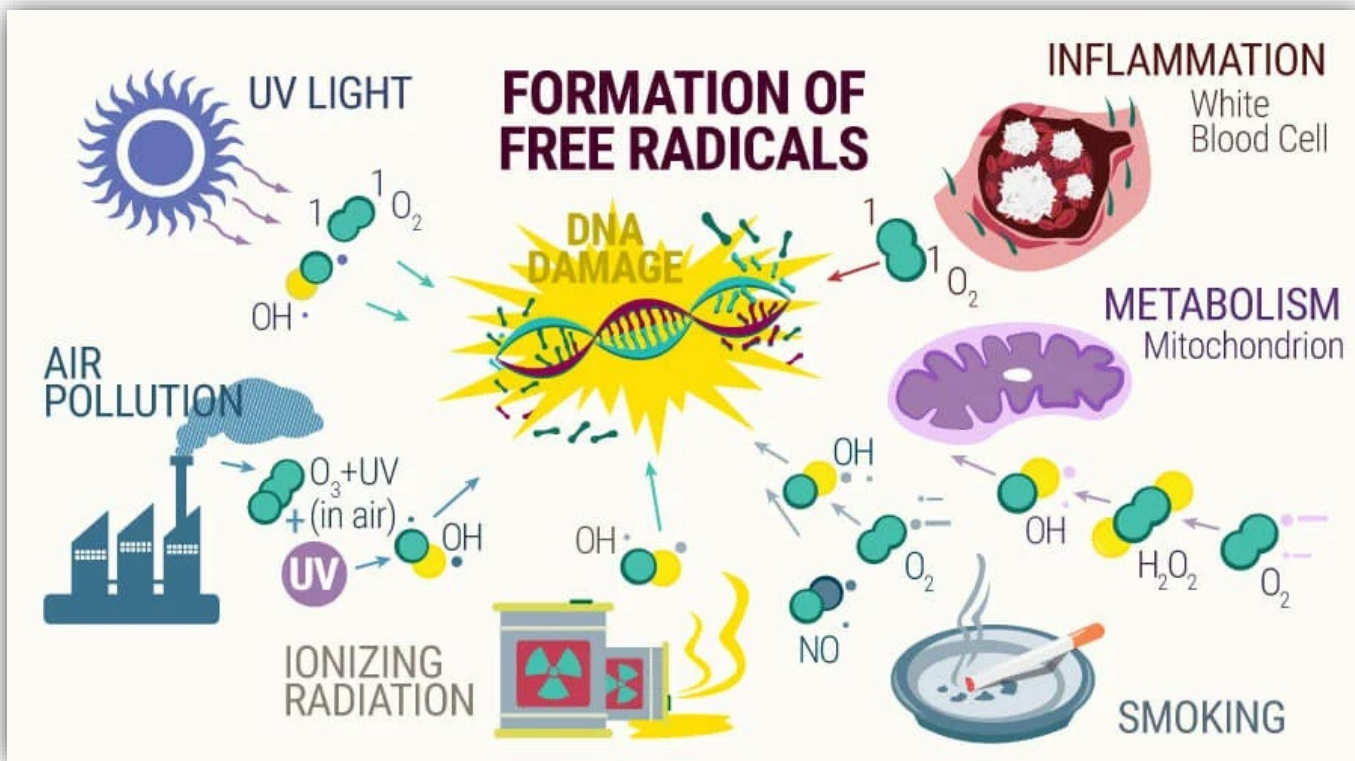
AIM OF THE STUDY



ANTIOXIDANT PROPERTIES

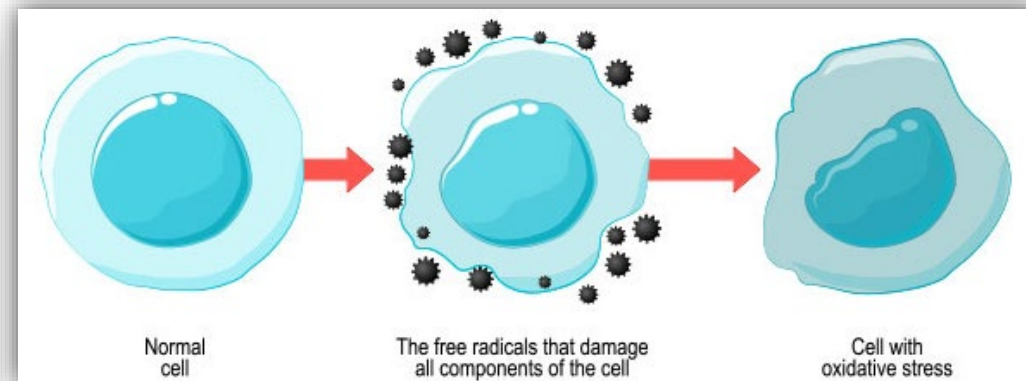
SEAWEEDS – HOW THEY AFFECT THE OXIDATIVE STRESS

FREE RADICALS FORMATION



Rajendran et al. 2013

OXIDATIVE STRESS



EXPERIMENT II

INTRODUCTION

ANTIOXIDANT PROPERTIES



Antioxidant

SEAWEEDS – THEIR ANTIOXODANT PROPERTIES



EXPERIMENTAL DESIGN

Seaweeds extracts were tested with:
3 extracts (AN, PP and 1:1 ANPP) ×
5 doses (0, 1, 50, 200, 500, 600 mg/mL).

1 Extract preparation



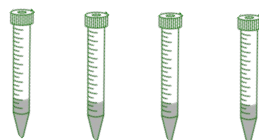
2 Extraction of the powders



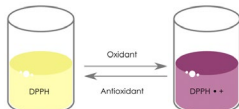
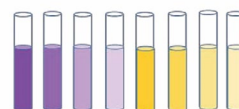
3 DPPH method



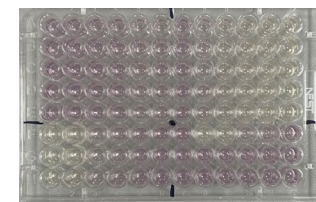
Add 3.9 mL of
DPPH solution



Incubate 30-mins in the dark



Add 100 μ L/well of solution
(in 96-well plates)



4

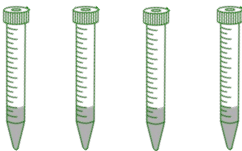


ABSORBANCE
MEASUREMENT

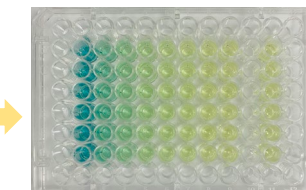
3 RPA method



Add:
2.5 mL 1% potassium ferricyanide,
2.5 mL 10% trichloroacetic acid,
0.5 mL 0.1% ferric chloride.



Incubate mixture at 50°C
for 20. minutes



Add 100 μ L/well
in a transparent
96-well plate

Data were analyzed
(SAS 9.4)

sas

METHODOLOGY

ANTIOXIDANT PROPERTIES

EXPERIMENT II



SEAWEEDS – THEIR ANTIOXODANT PROPERTIES

- Brown seaweed AN, and its extract mixes (ANPP) **demonstrated the highest dose** response starting from 50 mg/mL.

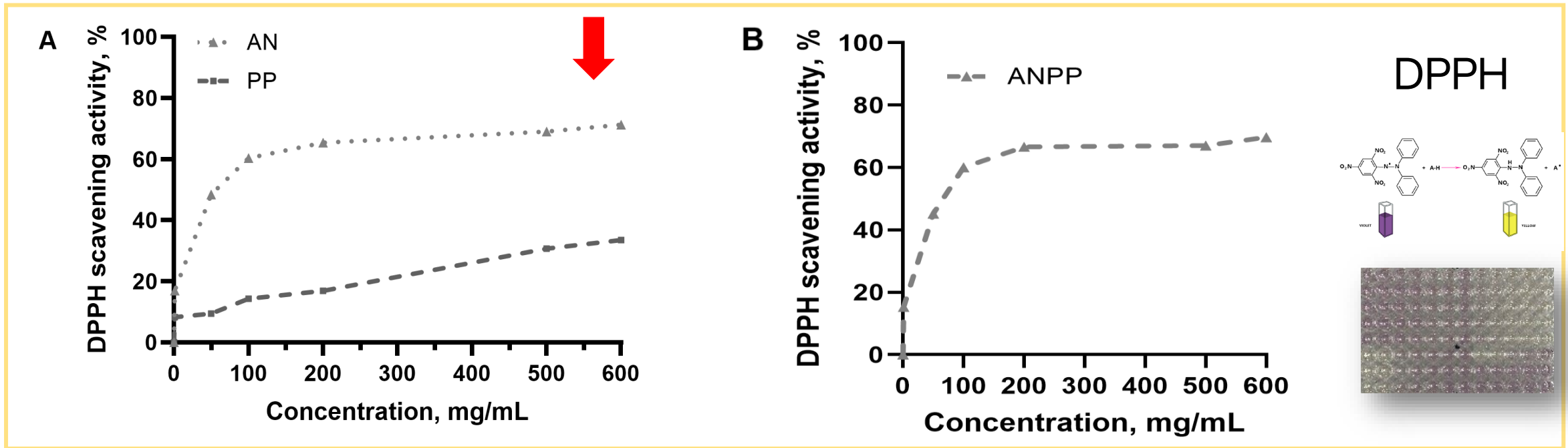


Fig. 4. Dose response of (A) *Ascophyllum nodosum* (AN) and *Palmaria palmata* (PP) algae species and their 1:1 extract mixes (B) using the 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Data are presented as the mean of 5 observations.

RESULTS



ANTIOXIDANT PROPERTIES

EXPERIMENT II

- Brown seaweed AN, and its extract mixes (ANPP) also **demonstrated the highest dose-response.**

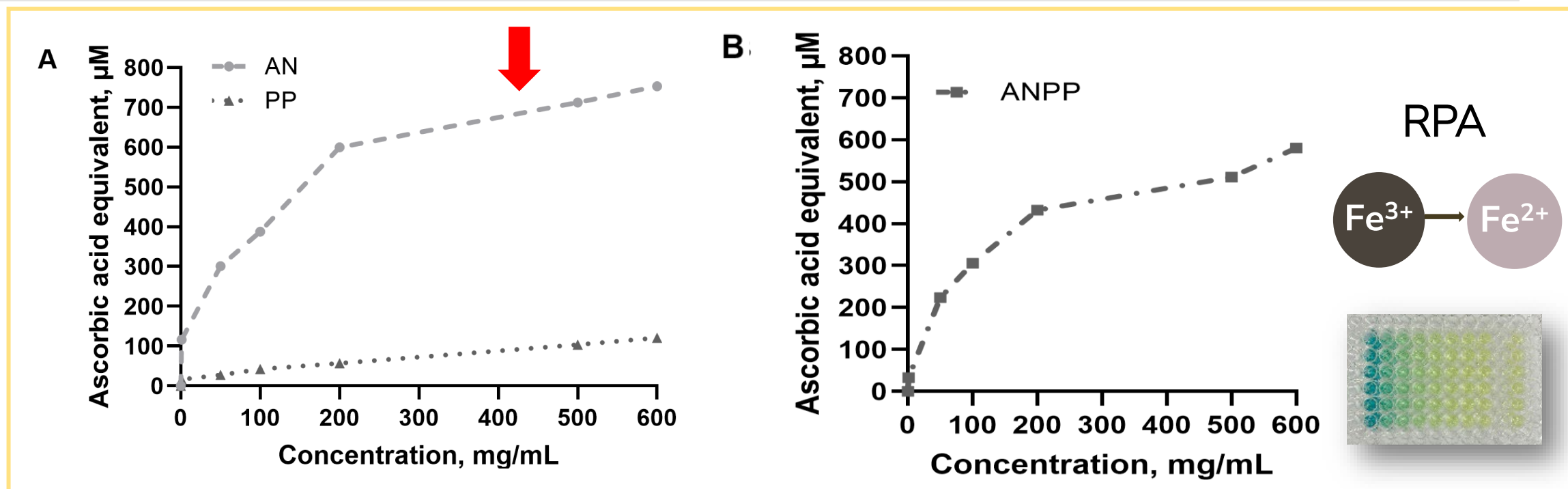
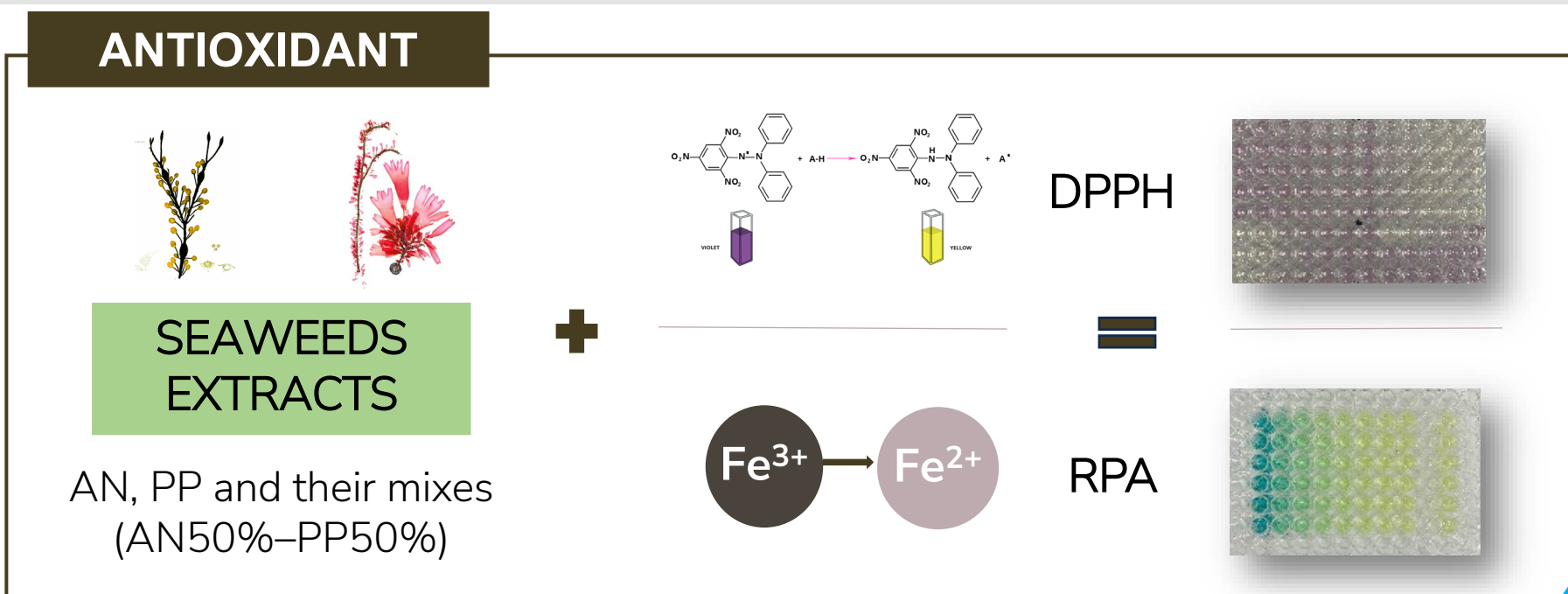


Fig. 5. Dose response of (A): *Ascophyllum nodosum* (AN) and *Palmaria palmata* (PP) algae species and (B): their 1:1 extract mixes using reducing power assay. Data are presented as the mean of 5 observations.

SEAWEEDS – THEIR ANTIOXODANT PROPERTIES

In this study, the brown macroalgae *Ascophyllum nodosum* extract and their mixes extracts were the most effective in terms of antioxidant activity in both assays.



EXPERIMENT II

CONCLUSION

ANTIOXIDANT PROPERTIES

SEAWEED – TAKE HOME MESSAGE

*Between animal and human medicine
there are no dividing lines—nor should there be.*

Rudolf Virchow, 1856

SUSTAINABILITY

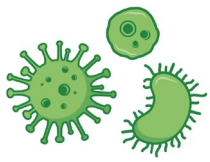


www.cdc.gov/onehealth

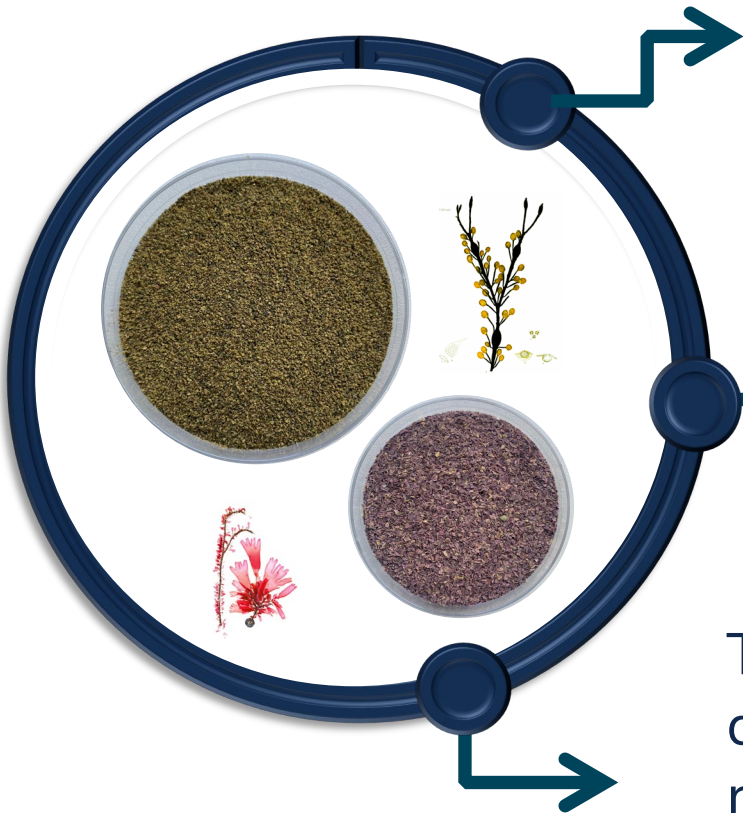


CONCLUSION

* EFSA (2017).
* ONZ, the 2030 Agenda for Sustainable Development (2015).



SEAWEEDS – TAKE HOME MESSAGE



Tested seaweed extracts, particularly from *Ascophyllum nodosum*, **showed strong antioxidative activity**, suggesting they may reduce oxidative stress.



Tested algae, **especially AN**, were able to significantly decrease the *Escherichia coli* growth. Thus, these seaweed extracts are able to **serve as antibacterial agents**.



These seaweeds could play a key role in the development of new **functional nutritional strategies** to reduce antibiotic overuse in swine farming, and **enhance sustainability**.



The sustainable development of livestock is a key.

CONCLUSION

ACKNOWLEDGMENTS



scientific reports



Assessment of the antibacterial and antioxidant activities of seaweed-derived extracts

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³ Department of Animal Science, University of California, 2251 Meyer Hall, One Shields Ave, Davis, 95616, CA, United States.

⁴ Department of Plant Experimental Biology and Biotechnology, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland.

PASIFIC



Ministry of Science and Higher Education
Republic of Poland

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Prof. Artur Jóźwik



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VETERINARIA E SCIENZE ANIMALI



UNIVERSITÀ DEGLI STUDI
DI MILANO



Dr Matteo Dell'Anno

Prof. Luciana Rossi



THANK YOU FOR YOUR ATTENTION!



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Maria Skłodowska-Curie Actions



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