

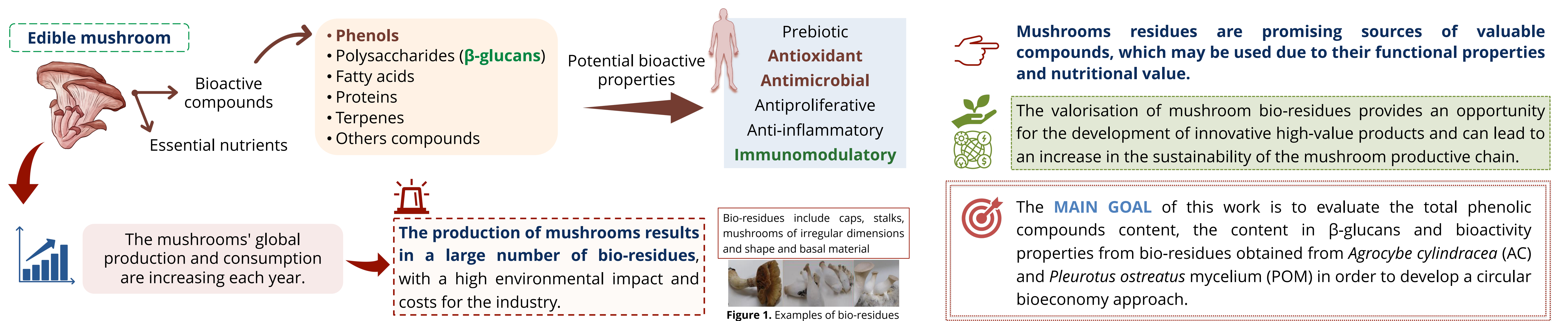
Antibacterial and antioxidant potential of aqueous extracts of bio-residues from *Agrocybe cylindracea* and *Pleurotus ostreatus* mycelium

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Introduction & Objectives



Methods

A. cylindracea bio-residues and *P. ostreatus* mycelium were provided by Voz da Natureza, Lda and were cleaned and frozen after harvest. The extraction was performed using water as the only solvent, in order to obtain a process as green as possible, with minimal costs and easily scaled up at industrial level. The aqueous extracts were obtained according to two different methods (1 and 2).

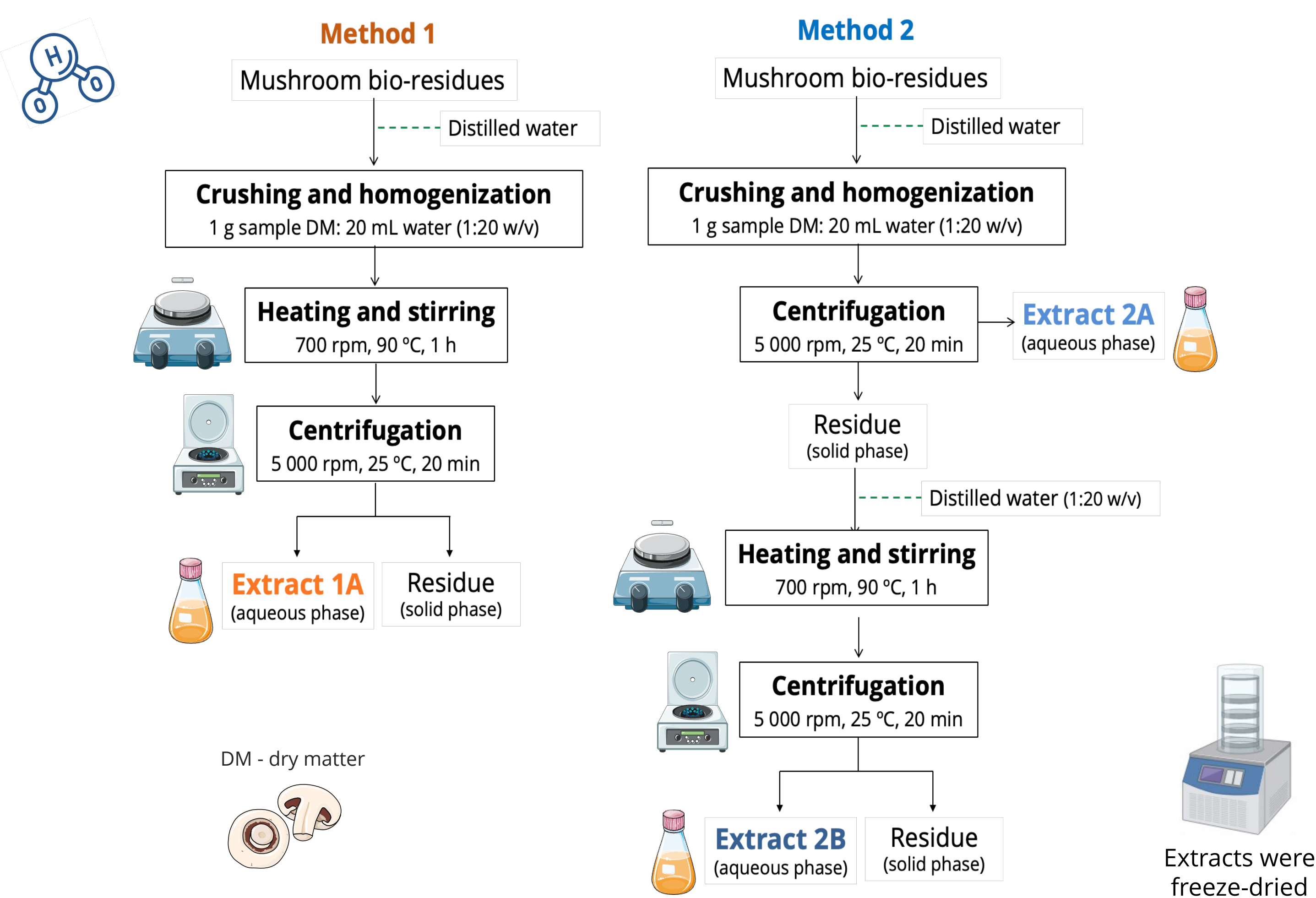


Figure 2. Extraction methods.

ANALYSIS

- Total phenolic content: **Folin-Ciocalteu colourimetry method**
 - Antioxidant activity: **ABTS, DPPH and ORAC** assays
 - β-glucans content: **"β-Glucan Assay Kit Yeast & Mushroom"** assay kit (Megazyme)
 - Antibacterial activity: determination of **minimum inhibitory concentration (MIC)** through microdilution method and the **rapid p-iodonitrotetrazolium chloride (INT) colourimetric assay**
 - Cytotoxicity: **PrestoBlue**
 - Mutagenicity: **Ames Test**
- **STATISTICAL ANALYSIS:** one-way ANOVA test ($p < 0.05$) was used to compare the mean of extracts from *A. Cylindracea*.

Results

Total phenolic compounds (TPC) content and antioxidant activity

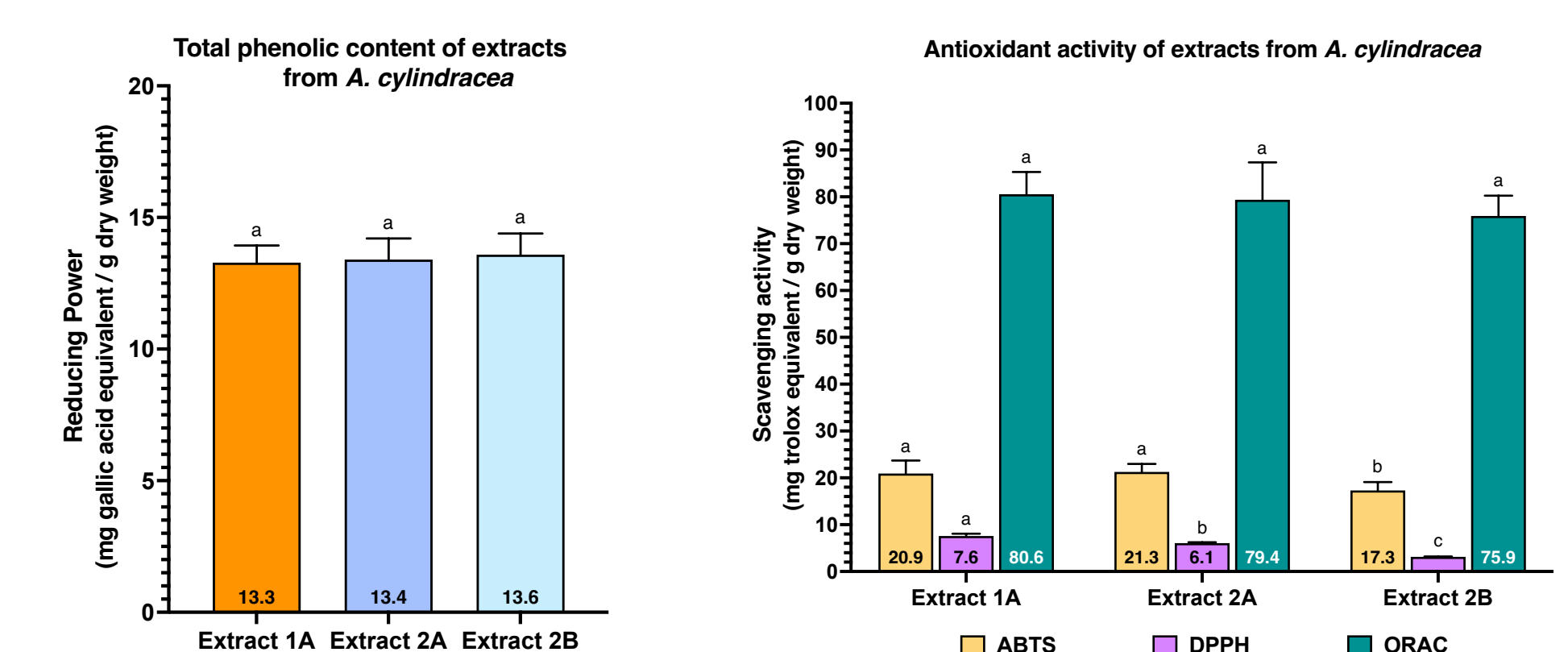


Figure 3. Total phenolic content. Results are the means of three determinations ± standard deviation. Different letters indicate a significant difference ($p < 0.05$) between extracts from *A. cylindracea*.

Figure 4. Antioxidant activity (ABTS, DPPH and ORAC assays). Results are the means of three determinations ± standard deviation. Different letters in the column with the same colour are significantly different ($p < 0.05$) between extracts from *A. cylindracea*.

Table 1. TPC and antioxidant activity of extracts from *P. ostreatus* mycelium (mean ± SD).

	Extract POM
Folin-Ciocalteu (mg gallic acid equivalent / g dry weight)	10.03 ± 0.08
ABTS assay (mg ascorbic acid equivalent / g dry weight)	4.14 ± 0.52
DPPH assay (mg trolox equivalent / g dry weight)	3.00 ± 0.55

All extracts were good sources of natural phenolic compounds. In general, the extracts from AC showed higher antioxidant capacity than extract from POM.

β-glucans

Table 1. Glucan content (all glucans, α-glucans, β-glucans) of bio-residues and aqueous extracts from *A. cylindracea* and *P. ostreatus* mycelium (mean ± SD). All results are expressed on a g/100g dry weight basis.

	Total Glucan (g/100g dwb)	α-Glucan (g/100g dwb)	β-Glucan (g/100g dwb)
Extract POM	1.01 ± 0.07	0.89 ± 0.05	0.12 ± 0.00
AC Bio-residues	43.53 ± 0.73 ^a	10.37 ± 0.14 ^a	33.16 ± 0.87 ^a
AC Extract 1A	12.38 ± 0.24 ^b	0.55 ± 0.01 ^{ab}	11.83 ± 0.23 ^b
AC Extract 2A	8.47 ± 0.28 ^b	0.34 ± 0.01 ^b	8.13 ± 0.27 ^b
AC Extract 2B	6.31 ± 0.10 ^b	0.38 ± 0.01 ^{ab}	5.93 ± 0.08 ^b

In each column different letters (a-d) mean significant differences ($p < 0.05$).

β-glucans represent 4–13% of the total dietary fiber. The **POM extract had more α-glucan content than β-glucans. Extract 1A has a higher β-glucan content**, which allows for predicting a potential natural source of these compounds with immunological and prebiotic activity.

Toxicity

The extract of *P. ostreatus* mycelium (10 mg/ml) did not have a genotoxic effect. Regarding the extracts from *A. cylindracea* (40 mg/ml), only extract 1A showed cytotoxicity and genotoxic effects. In this sense, extracts 2A and 2B are expected to be safe for application in food products.

Antibacterial activity

Table 2. MIC values (mg/ml) of extracts from *A. cylindracea* against Gram-positive bacteria.

	<i>Bacillus cereus</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>
Extract 1A	> 40	10	> 40
Extract 2A	> 40	> 20	> 40
Extract 2B	> 40	> 20	> 40

The antimicrobial activity of the POM extract was evaluated through growth curves of *E. coli*, *P. aeruginosa* and *S. aureus*. There was only inhibition of growth in *E. coli* (28% after 24h).

In general, the aqueous extracts of *A. cylindracea* did not inhibit bacterial growth.

Table 3. MIC values (mg/ml) of extracts from *A. cylindracea* against Gram-negative bacteria.

	<i>Salmonella enterica</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>P. fluorescens</i>	<i>P. tolaasii</i>	<i>P. agarici</i>	<i>Yersinia enterocolitica</i>
Extract 1A	40	> 40	> 40	> 40	> 40	> 40	40
Extract 2A	20	> 40	> 40	> 40	> 40	> 40	20
Extract 2B	> 40	> 40	> 40	> 40	> 40	> 40	40

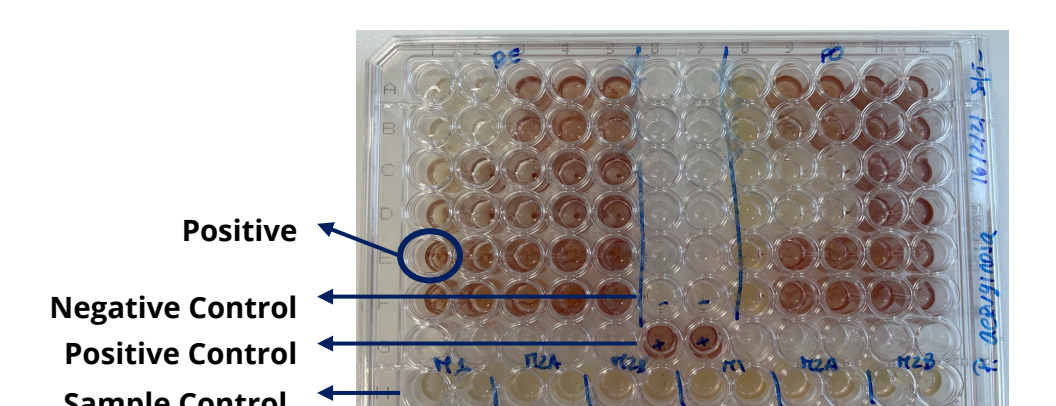


Figure 4. Example of an antimicrobial test microplate.

Conclusions

According to the result, these aqueous extracts of *A. cylindracea* bio-residues can be considered a natural source of phenolic compounds, compounds with antioxidant activity and β-glucans. The POM extract should continue to be investigated to learn more about its bioactive potential. Thus, the valorisation of these bio-residues is viable by developing "green" value-added products with great potential for application in nutraceutical and food products such as food conservatives or food coatings, contributing to a circular economy.

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