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Exploring Beer By-products as Novel Antibacterial Ingredients for Health Care Products

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INTRODUCTION & AIM

Beer is a widely consumed carbonated beverage made from natural ingredients, including malted cereal, hops, yeast and water[1]. It is rich in nutrients and contains carbohydrates, minerals, vitamins, amino acids and polyphenols[2]. The brewing process, however, generates a significant amount of solid waste, including hot trub, a slurry of entrained wort, hop particles and mainly unstable high molecular weight colloidal proteins that coagulate during the boiling of the wort[3]. Given the environmental impact of agro-industrial waste, finding sustainable methods to reuse these wastes by transforming them into bio-products is crucial. The aim of this study was to assess the potential of converting these by-products into biologically active extracts, suitable for use as functional ingredients in cosmetic and pharmacological formulations.

METHOD

Hot trub was supplied by a brewery named Musa, Lisbon, Portugal.

The material was subjected to the drying process at 45ºC in lab, until dry and stored at room temperature.

Figure 1. Hot trub after drying process.

1. Preparation of the Hot trub extracts

Three different extractions were performed: Two extractions using the Soxhlet method - Hydroalcoholic (ethanol/water 70:30), and Alcoholic (99.9% ethanol) during 90 min at high temperature; and one more Hydroalcoholic Extraction (ethanol/water 70:30) by maceration in agitation (900 rpm) at room temperature for 24 h. All the extracts were evaporated, and the resulting extracts were stored in Eppendorf tubes and frozen.

Figure 2. Soxhlet Extraction Equipment.

Figure 3. Maceration Extraction Method.

Figure 4. Rotary Evaporator Equipment.

2. *In vitro* Antimicrobial Activity

In vitro antimicrobial activity was determined according to the Clinical and Laboratory Standards Institute.[3] All assays were performed in triplicate and negative controls were also included.

A) Well Diffusion Method

The antimicrobial growth inhibition was carried out using *in vitro* conventional well diffusion method and was employed for the initial assessment of the antimicrobial potential of the extracts. A standardized saline bacterial suspension, corresponding to 10^8 CFU/mL was prepared and used to inoculate the Mueller-Hinton Agar plates. Wells with 6.0 mm diameter were, then, made and in each of them 50 µL of extract at 50 mg/mL in DMSO was added. The plates were kept in an incubator at 37°C during 24 h. The antimicrobial activities were determined by measuring, in mm, the diameter of the growth inhibition zone.

B) Determination of Minimum Inhibitory Concentrations

The minimal inhibitory concentration (MIC) was determined by the broth microdilution method in 96-well microtiter plates. The extract was prepared at 100 mg/mL in DMSO and a dilution of 1:10 was introduced in the first line, followed by a series of 2-fold dilutions made in the plate in the Mueller-Hinton broth. A standardized saline bacterial suspension, corresponding to 10^8 CFU/mL, was prepared and used to inoculate microtiter plates. After incubation at 37°C for 24 h, turbidity of the broth in the wells was observed and the minimal inhibitory concentration was defined as the lowest broth concentration of extracts at which no visible growth could be detected.

RESULTS & DISCUSSION

The conventional well diffusion and the broth microdilution methods were employed for the assessment of the antibacterial potential of the Hot trub extracts against Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 11778), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus (MRSA)* (CIP 106760), *Streptococcus mitis* (NCIMB 13770), *Streptococcus mutans* (ATCC 25175), *Streptococcus pyogenes* (ATCC 12384) and *Enterococccus faecalis* (ATCC 29212); Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739); and Yeast: *Candida albicans* (ATCC 29212).

Table 1. Antimicrobial activity in diffusion assay for the Hot trub extracts.

Inhibition zone (mm)	AES	HES	HEM	NC*	Van**	Ofl**	Ket**
S.aureus	14.25±1.89	14.75±1.71	12.50±1.00	-	28.00±0.00	n.d.	n.d.
B.cereus	15.75±0.96	15.75±0.96	14.50±0.58	-	27.00±1.41	n.d.	n.d.
S.epidermidis	22.25±0.50	22.75±0.96	16.75±0.96	-	18.50±0.70	n.d.	n.d.
P.aeruginosa	-	-	-	-	n.d.	-	n.d.
E.coli	-	-	-	-	n.d.	19.50±0.70	n.d.
S.aureus (MRSA)	14.50±1.00	14.50±1.29	13.00±1.15	-	26.00±1.41	n.d.	n.d.
S.mitis	13.00±2.16	12.00±1.63	10.33±0.58	-	22.00±0.00	n.d.	n.d.
S.mutans	13.25±1.71	13.25±0.96	13.00±1.41	-	19.00±1.41	n.d.	n.d.
S.pyogenes	13.00±4.08	13.00±3.46	-	-	29.00±4.24	n.d.	n.d.
E.faecalis	18.75±1.71	19.75±1.71	17.25±1.71	-	22.50±0.71	n.d.	n.d.
C.albicans	-	-	-	-	n.d.	n.d.	27.00±1.41

AES - Alcoholic Extract Soxhlet; HES - Hydroalcoholic Extract Soxhlet; HEM - Hydroalcoholic Extract Maceration; *Negative Control; **Positive Controls; Van - Vancomycin ; Ofl - Ofloxacin; Ket - Ketaconazole; n.d.- not detected; - inactive

Table 2. Minimum Inhibitory Concentration (MIC) (µg/mL).

Minimum inhibitory concentration (MIC)	AES	HES	HEM	NC*	Van**	Ofl**	Ket**
S.aureus	625	625	2500	> 10 000	1.56	n.d.	n.d.
B.cereus	313	313	1250	> 10 000	0.78	n.d.	n.d.
S.epidermidis	625	625	2500	> 10 000	1.56	n.d.	n.d.
Paeruginosa	> 10 000	> 10 000	> 10 000	> 10 000	n.d.	0.625	n.d.
E.coli	> 10 000	> 10 000	> 10 000	> 10 000	n.d.	0.039	n.d.
S.aureus (MRSA)	625	1250	2500	> 10 000	1.56	n.d.	n.d.
S.mitis	5000	10 000	> 10 000	> 10 000	3.12	n.d.	n.d.
S.mutans	> 10 000	> 10 000	5000	> 10 000	12.5	n.d.	n.d.
S.pyogenes	5000	10 000	> 10 000	> 10 000	3.12	n.d.	n.d.
E.faecalis	5000	5000	10 000	> 10 000	3.12	n.d.	n.d.
C.albicans	10 000	10 000	10 000	> 10 000	n.d.	n.d.	0.039

AES - Alcoholic Extract Soxhlet; HES - Hydroalcoholic Extract Soxhlet; HEM - Hydroalcoholic Extract Maceration; *Negative Control; **Positive Controls; Van - Vancomycin ; Ofl - Ofloxacin; Ket – Ketaconazole; n.d.- not detected; - inactive

The minimum inhibitory concentration (MIC) and inhibition zones values obtained showed significant inhibitory effect against the gram-positive bacteria tested, with the Soxhlet extracts presenting the best results of inhibition for both hydroalcoholic and alcoholic extracts. In both methods the microorganisms *S. epidermidis* and *B. cereus* showed the best antibacterial activity, with an MIC of 625 and 313 µg/mL, respectively (Table 2).

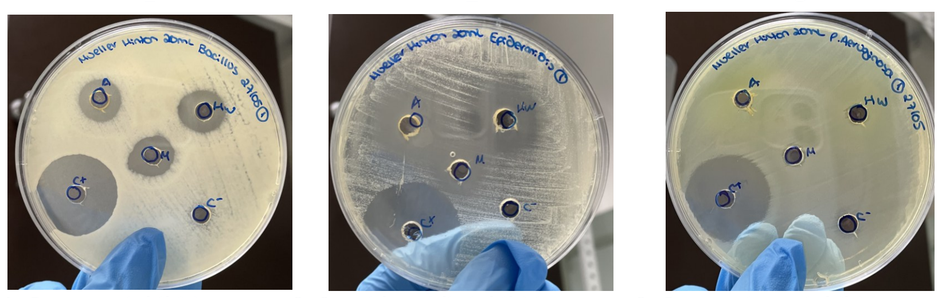

Figure 5. 1- *B. cereus* inhibition zone; 2- S. epidermidis inhibition zone; 3- *P. aeruginosa* inhibition zone; A - Alcoholic Extract Soxhlet; HW - Hydroalcoholic Extract Soxhlet; M - Hydroalcoholic Extract Maceration; C^- - Negative Control; C^+ - Positive control.

CONCLUSION

The MIC and inhibition zones values obtained showed significant inhibitory effect against the Gram-positive bacteria tested, with the Soxhlet extracts presenting the best results of inhibition for both hydroalcoholic and alcoholic extracts. The tested extracts, seems to be a promising low-cost antibacterial agent that can be incorporated in health care products.

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