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SHORT COMMUNICATION

FIRST DATA ON THE ANTIMICROBIAL ACTIVITY OF *YUCCA FILAMENTOSA* L. BARK EXTRACTS

M. BONONI, G. GUGLIELMI[1], P. ROCCHI[1] and F. TATEO*
Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano,
Via Celoria 2, 20133 Milano, Italy
[1]Rocchi dr. Eugenio s.r.l., Via J.F. Kennedy 3, 40053 Bazzano, BO, Italy
*Corresponding author: Tel. +39 02 50316540, Fax +39 02 50316539,
email: fernando.tateo@ .it

ABSTRACT

The aim of this study was to investigate the antimicrobial activity of two *Yucca filamentosa* L. extracts produced by hydro-alcoholic extraction under different operational conditions. The extracts were concentrated and de-alcoholised, and were added to media to test their effects on target microorganisms. Both extracts displayed equivalent specific growth inhibitory activity against *Saccharomyces cerevisiae*. No other antimicrobial activity was found. This anti-fermentative activity may open the doors to new possibilities for using *Yucca filamentosa* L. extracts in the food industry. These findings are of particular interest, since yucca extracts are already classified as being generally recognized as safe (GRAS).

- Keywords: *Yucca filamentosa* L., antimicrobial activity, yucca extracts -

INTRODUCTION

The various *Yucca* species (fam. Asparagaceae, sub-fam. Agavoideae) are tropical or sub-tropical plants with a tree-like growth habit. They are found in arid or semi-arid South-Western regions of the US and Mexico (PELLMYR, 2003). In traditional medicine of the Native Americans, yucca-derived extracts were used against a variety of diseases, including arthritis and rheumatism (MUKHERJEE *et al.*, 2001); these extracts have GRAS (generally recognized as safe) status and are therefore accepted by the US FDA for use in humans. Yucca powder and sap are derived from the logs of the plant; such extracts can be produced by mechanical squeezing and subsequent evaporation of the sap, and are widely used in food, cosmetics, and pharmaceutics (MATTIA and COLUZZI, 2005). Yucca powder and extracts are also used as animal feed additives due to their beneficial anti-inflammatory effects (WENZIG *et al.*, 2008), which have been linked to their high content of phenolic compounds.

Many studies have also examined the saponin-rich extracts of different *Yucca* species for antibacterial activity (CHEEKE *et al.*, 2006; FAVEL *et al.*, 2005; KILLEN *et al.*, 1998; KOWALCZYK *et al.*, 2011; MILGATE and ROBERTS, 1995; MIYAKOSHI *et al.*, 2000; MONTORO *et al.*, 2008; PIACENTE *et al.*, 2005; WENZIG *et al.*, 2008). Saponins are glycoside compounds of a fat-soluble nucleus (aglycone) that can be either a triterpenoid (C_{30}), as in soybean, alfa-alfa, quillaja, and guar (HASSAN *et al.*, 2010); or an alkaloid steroid (C_{27}), as in yucca, tomato, and oats. One or more side chains or water-soluble sugars (glycone) are attached through ester linkages to the aglycone nucleus at different carbon sites (KANEDA *et al.*, 1987). Saponins have shown haemolytic (KHALIL and EI-ADAWY, 1994) and antibacterial activities (SEN *et al.*, 1998), but not all saponins have the same biological activities (HASSAN *et al.*, 2010, KANEDA *et al.*, 1987). It has been demonstrated that yucca bark accumulates polar bidesmosidic saponins; the bark powder samples are dominated by high polarity, while steroidal glycosides with mid- and short-length saccharide chains are predominant in the steam.

A large number of papers have determined that *Yucca schidigera* contains steroidal saponins with anti-yeast activity, and therefore such extracts have been investigated for use as new agents to prevent food deterioration (MIYAKOSHI *et al.*, 2000). Our preliminary experiments show similar properties exhibited by root extracts of *Yucca filamentosa* L. from the USA, also studied previously addressing the importance of chemistry and geographic variation of floral scent (SVENSSON *et al.*, 2005). In the present study, we tested the efficiencies of producing *Yucca filamentosa* L. extract using different extraction solvents, and then tested the antimicrobial activities of these extracts *vs.* several target microorganisms. We found evidence that the extracts had considerable microbicidal activity exclusively against *Saccharomyces cerevisiae* and not against other target microorganisms.

MATERIAL AND METHODS

Extract production for microbiological testing

During the extraction, and particularly during separation of the solid waste, we attempted to avoid introducing technologically differentiated parameters for the various extracts by adopting the simple spontaneous drainage, without affecting by pressure the release of active principles from solid. The dry vegetable material of *Yucca filamentosa* L. was cut into pieces with an average diameter of 10 mm. Preliminary extractions were performed under varying conditions, in order to identify the parameters that produced the best results in terms of percentage of the total soluble solids in the extracts.

Extractions were performed by seven days of infusion of dry materials with solvent in a solid:liquid ratio of 1:6.7; the extraction mixture was shaken three times per day. Three different solvents were used: (A) water, (B) water/95% ethanol (50:50 v/v), and (C) 95% ethanol. In theory, derivation using different types of solvents could result in the extracts having different compositions of the active substances.

Extraction efficiencies were measured (Table 1). Solvent B was determined to produce the best extractive yield. A new extract was produced using solvent B, this time cutting the dry yucca to pieces with an average diameter of 1-2 mm. This extraction product was concentrated in a rotovapor (2:1); the obtained product was named extract B and contained a concentration of total soluble solids equal to 14.0 g 100 mL^{-1}. We also combined the original extracts produced with solvents A, B, and C, in a ratio of 1:1:1. This mixture was concentrated in a rotovapor (4:1) to produce an extract with total soluble solids concentration of 14.2 g 100 mL^{-1}, similar to that of the concentrated extract B. Both concentrated products had a residual ethanol content of no more than 0.01%. The concentrated extracts B and ABC were next tested for their activity on microorganisms.

Microbiological verifications

For testing the antimicrobial activity of the yucca extracts, the following species were used as target microorganisms: *Aspergillus brasiliensis* (NCPF 2275/ATCC 16404), *Escherichia coli* (NCTC 12241/ATCC 25922), *Staphylococcus aureus* (NCTC 12981/ATCC 25923), *Listeria monocytogenes* (NCTC 11994), *Bacillus cereus* (NCTC 7464/ATCC 10876), and *Saccharomyces Cerevisiae* (NCPF 3178). These strains

Table 1 - Preliminary results of testing three different extraction solvents for *Yucca filamentosa* L.

	Yucca (g)	95% EtOH (mL)	H_20 (mL)	Extract (mL)	Total Soluble Solids (g 100 mL^{-1})
Solvent A	888	6,000	---	4,330	0.56
Solvent B	878	3,000	3,000	3,800	5.32
Solvent C	880	---	6,000	2,400	3.58

were obtained from TCS Biosciences Ltd. (Botolph Claydon, Buckingham, UK), and are the standards used for quality control testing in the Laboratory "Rocchi dr. Eugenio s.r.l", Bazzano (Bologna, Italy).

Solid media used included Sabouraud dextrose agar (Biokar diagnostic) for *Aspergillus brasiliensis* and *Saccharomyces cerevisiae*, and tryptone soy agar (Oxoid) for all other target microorganisms. Each solid medium was prepared in the following different versions: undiluted, with the addition of 1 mL ABC extract 100 mL^{-1} of medium, and with the addition of 1 mL B extract 100 mL^{-1} of medium. Testing verified that the addition of extracts did not significantly modify the pH of the medium; the pH remained in the interval of 7.1 ± 0.02 for tryptone soy agar, and of 5.5 ± 0.05 for Sabouraud dextrose agar.

The stock suspensions for the inoculum were produced by collecting a loopful (with a 1 µL loop) of target microorganisms from slant cultures, and dispersing the microorganisms into 10 mL of sterile Ringer 1\4 solution (Biokar diagnostic). From these stock suspensions, decimal dilutions were prepared up to the third. Then 50 µL of the third and the second decimal dilutions were used to inoculate four replica plates, undiluted on the surface of the media with a Don Whitley spiral plater. Incubation conditions were chosen in accordance with the nature of each target microorganisms as follows: *Aspergillus brasiliensis*, 25°C for 5 days; *Escherichia coli*, 37°C for 24 hours; *Staphylococcus aureus*, 37°C for 48 hours; *Listeria monocytogenes*, 37°C for 48 hours; *Bacillus cereus*, 30°C for 48 hours; *Saccharomyces cerevisiae*, 25°C for 5 days.

Table 2 - Results concerning growth values as cfu mL^{-1} derived from sowing stock suspension on undiluted media and media modified with two yucca extracts (ABC and B). Counts on tryptone soy agar (t.s.a.) and Sabouraud dextrose agar (s.a.) media are reported. The values are the average of four replicas, calculated according to ISO 8199:2005 Standard (8.4.2); standard deviation (S) and *p* values are reported for the significant growth reduction of *S. cerevisiae.*

	Medium	cfu mL^{-1}	95% Confidence Interval (cfu mL^{-1})		Log cfu mL^{-1}	95% Confidence Interval (Log cfu mL^{-1})			
Bacillus	t.s.a.	1.456×10^6	1.451×10^6	1.461×10^6	6.163	6.162	6.165		
cereus	t.s.a. + ABC	1.475×10^6	1.470×10^6	1.480×10^6	6.169	6.167	6.170		
	t.s.a. + B	1.341×10^6	1.337×10^6	1.346×10^6	6.127	6.126	6.129		
Escherichia	t.s.a.	23.249×10^6	23.159×10^6	23.339×10^6	7.366	7.365	7.368		
coli	t.s.a. + ABC	34.424×10^6	34.290×10^6	34.558×10^6	7.537	7.535	7.539		
	t.s.a. + B	24.324×10^6	24.236×10^6	24.411×10^6	7.386	7.384	7.388		
Staphylococcus	t.s.a.	4.236×10^6	4.221×10^6	4.250×10^6	6.627	6.625	6.628		
aureus	t.s.a. + ABC	4.252×10^6	4.237×10^6	4.268×10^6	6.629	6.627	6.630		
	t.s.a. + B	5.026×10^6	5.005×10^6	5.047×10^6	6.701	6.699	6.703		
Listeria	t.s.a.	26.424×10^6	26.322×10^6	26.526×10^6	7.422	7.420	7.424		
monocytogenes	t.s.a. + ABC	20.999×10^6	20.916×10^6	21.082×10^6	7.322	7.320	7.324		
	t.s.a. + B	28.126×10^6	28.013×10^6	28.238×10^6	7.449	7.447	7.451		
Aspergillus	s.a.	31.500×10^3	30.706×10^3	32.294×10^3	4.498	4.487	4.509		
brasiliensis	s.a. + ABC	29.500×10^3	28.732×10^3	30.268×10^3	4.470	4.458	4.481		
	s.a. + B	33.500×10^3	32.681×10^3	34.319×10^3	4.525	4.514	4.536		
Saccharomyces	s.a.	68.333×10^4	67.358×10^4	69.307×10^4	5.835	5.828	5.841	S	p
cerevisiae	s.a. + ABC	3.000×10^2	2.452×10^2	3.548×10^2	2.477	2.390	2.550	0.12	<0.0005
1st test	s.a. + B	2.500×10^2	2.000×10^2	3.000×10^2	2.398	2.301	2.477	0.17	<0.0005
Saccharomyces	s.a.	19.365×10^5	19.306×10^5	19.425×10^5	6.287	6.286	6.288	S	p
cerevisiae	s.a. + ABC	5.500×10^3	5.265×10^3	5.735×10^3	3.740	3.721	3.758	0.10	<0.0005
2nd test	s.a. + B	25.250×10^3	24.748×10^3	25.752×10^3	4.402	4.394	4.411	0.29	<0.0005

RESULTS AND CONCLUSIONS

At the end of the incubation, a relative count was performed for each microorganism, enabling comparison of the growth on control media with that on media modified by adding the two extracts, ABC and B. The calculations took into account the results obtained from all dilutions and replicas sown with each target microorganism, and applied the formula as per the ISO 8199:2005 standard at point 8.4.2. The 95% confidence interval was also determined for each count, as indicated by the aforementioned ISO standard. Preliminary sterility evaluations were made on media loaded with the extracts ABC and B, and showed that the extracts had negligible bacteria content of 560 cfu mL^{-1} for the ABC extract and 640 cfu mL^{-1} for the B extract. Such values confirm the non-existence of interference from microbial content peculiar to the extracts.

Table 2 reports the results for each target microorganism; values express the growth as colony forming units (cfu) per mL, derived from sowing the stock suspensions on either the undiluted media or the media modified with the two examined yucca extracts. Table 2 also presents the data expressed in log cfu mL^{-1} and the 95% confidence intervals. Data show that yucca extracts had a significant microbial growth reduction effect only on *S. Cerevisiae*, statistically confirmed by *p*-values (<0.0005) as reported in Table 2; it is very clear that the effect of ABC and B extracts doesn't exist against other target microorganisms at the applied 1% concentration (no inhibition is readable on logarithmic scale). Data clearly show the effects of both extracts on *Saccharomyces cerevisiae* compared to the unaltered media; the activity of the two *Yucca filamentosa* L. extracts led to inhibition by more than 3 logarithmic scales. The activity is confirmed by a 2nd test performed ten days later: the results are reported in Table 2.

The Student's *t*-test was used to analyse the data, confirming that a normal distribution could be assumed. This statistical analysis showed that except in the case of *S. cerevisiae*, non-significant differences were detected in the target microorganism counts among the undiluted media and the media modified with extracts ABC and B. The statistically significant effect on *S. cerevisiae* growth was repeatable between the four replicas, in two quantitative tests performed using a basic medium from two different batches.

The present reported, specifically growth inhibitory activity of *Yucca filamentosa* L. extracts against yeast opens a wide range of possible applications for using these extracts in the food sector. Further studies should be performed to investigate the effectiveness of these extracts on other microorganisms with a fermentative metabolism.

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