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Antibacterial activity of *Borago officinalis* and *Brassica juncea* aqueous extracts evaluated *in vitro* and *in situ* using different food model systems

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ABSTRACT

The present study was undertaken to characterize the antibacterial activity of the aqueous extracts (AEs) obtained from the leaves of *Borago officinalis* L. and *Brassica juncea* L. The antagonistic activity was evaluated against several bacteria (42 strains of *Listeria monocytogenes*, 35 strains of *Staphylococcus aureus*, 38 strains of *Enterobacter* spp. and 18 strains of *Salmonella enterica*) commonly associated with foodborne diseases by paper disc diffusion method. The susceptibility to the plant extracts was strain specific. Thirty-five strains (7 *L. monocytogenes*, 11 *S. aureus*, 1 *S.* Enteritidis, 1 *S.* Veneziana, 7 *Enterobacter hormaechei*, 5 *Enterobacter cloacae*, 1 *Enterobacter sakazakii* and 2 *Enterobacter amnigenus*) were sensitive to both AEs. The activity of *B. juncea* AE towards the Gram-positive strains was generally higher than that observed for *B. officinalis* (45 and 22 strains inhibited by *B. juncea* and *B. officinalis*, respectively), while an opposite trend was registered against the Gram-negative strains (22 and 35 strains inhibited by *B. juncea* and *B. officinalis*, respectively). The highest inhibition was displayed by *B. juncea* AE against *E. sakazakii* 23A. *B. officinalis* AE showed the same minimum inhibitory concentration (MIC) (10 mg/mL) for the majority of the most sensitive strains, while the MIC of *B. juncea* AE was different for each bacterial species and the lowest concentration was registered to inhibit enterobacteria (3.1 mg/mL). After 1-year storage in different thermal conditions (room temperature, 4 °C and −20 °C), both AEs lost their inhibitory power. The extracts did not show cellular toxicity when tested against sheep erythrocytes. Hence, *B. officinalis* and *B. juncea* AEs were effective as natural antibacterial substances. AEs were tested *in situ* in three food model systems (meat, fish and vegetable) at two concentrations, but only when added at a concentration 10-fold higher than that showing definite efficacy *in vitro* (100 and 31 mg/mL for *B. officinalis* and *B. juncea*, respectively), they inhibited the growth of the sensitive strains, even though the cells were still viable after 24 h. The influence of AEs on the volatile organic compounds (VOCs) composition of the food models was analysed by gas chromatography/mass spectrometry. The different levels of alcohols, aldehydes, esters, hydrocarbons, ketones and phenol registered, showed a consistent effect of *B. officinalis* and *B. juncea* AEs on the VOCs of the food models. However, the sniffing assay found only *B. juncea* AE impacting consistently the final aroma of the food models.

1. Introduction

Several pathogens still represent a major public health problem in both developed and developing countries. *Salmonella* spp., *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and entero-pathogenic *Escherichia coli* cause over 90% of all cases of food poisoning (Friedman, Henika, & Mandrell, 2002; Wilson & Droby, 2000). *Enterobacter* spp. are also often responsible for human sporadic and epidemic cases associated with food matrices (Healy, Cooney, O'Brien, Iversen & Whyte, 2010). Moreover, *Staphylococcus aureus* that is responsible for the most frequent foodborne intoxication, is acquiring a new epidemiological dimension through the putative role of some foods, in particular chicken and pork, as a vehicle of livestock-associated methicillin resistant (MRSA) strains (Harrison et al. 2013).

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The extensive use of antimicrobials has driven increasing resistances among several bacterial species and, as a matter of fact, the efficacy of these inhibition compounds is seriously decreased (Lai, Tremblay, & Déziel, 2009; SCENIHR, 2010). In its opinion on antimicrobial resistance (European Commission, 1999), the Scientific Steering Committee recommended "the prudent use of antimicrobials". Thus, due to the increasing pressure of consumers and legal authorities, food processors, food researchers, and regulatory agencies are showing interest towards natural products with bactericidal activity. Several compounds found in plants, which have long been used as agents for food preservation, represent natural alternatives to chemicals for the maintenance or shelf-life extension of food products (Nychas, Tassou, & Skandamis, 2003).

Borage (*Borago officinalis* L.) is a hairy annual herbaceous plant of the Boraginaceae family native to Europe and North Africa widely spread in many Mediterranean countries. This plant is cultivated throughout the world and is used for folk medicinal purposes (Hassan Gilani, Bashir, & Khan, 2007), as well as for preparing beverages and salads (Branca, 2001). Plants and seeds of borage provide bio-active compounds (Bandoniene & Murkovic, 2002; Duke, 1992; Gudej & Tomczyk, 1996; Mhamdi, Wannes, Bourgou, & Marzouk, 2009) and, due to its content in gamma linolenic acid (GLA), borage is gaining increasing agricultural interest (El Hafid, Blade, & Hoyano, 2002).

Indian mustard (*Brassica juncea* L.) has been cultivated for centuries in many parts of Eurasia from which originates. It is commonly used as leafy vegetable or for its seeds that are widely used in many Countries as a traditional pungent spice, and a type of medicine. The pharmacological effects of *B. juncea* are due to the isothiocyanates it contains (Björkman, Klingen, Birch, Bones & Bruce, 2011). The essential oil of *B. juncea* is of high value and can be used to suppress the growth of various microorganisms (Yu, Jiang, Li, & Chan, 2003).

Since plants of Boraginaceae family and *B. juncea* are known to be effective *in vitro* against some human pathogens (Abolhassani, 2004; Ahmad, Mehmood, & Mohammad, 1998), it is important to deepen the knowledge on their inhibitory effects on the most common foodborne bacteria in order to develop strategies for future applications of plant extracts as biopreservative agents in foods. Hence, to extend the exploitation of *B. officinalis* and *B. juncea*, the aims of this study were: to investigate the antagonistic effect of their aqueous extracts against some common pathogenic bacteria *in vitro*; to determine their stability over time; to evaluate their cytotoxicity; to estimate their efficacy in food model systems (*in situ*); and to determine their impact on the volatile organic compounds of the food models.

2. Materials and methods

2.1. Plant material, preparation of aqueous extracts and bacterial strains

Plants of *B. officinalis* L. and *B. juncea* L. were grown during autumn-spring in the experimental field of the Department of Agricultural and Forest Science, University of Palermo (38°9′28″N, 13°20′3″E). Plants were collected at flowering and leaves were immediately separated, cleaned, washed and comminuted. Each aqueous extract (AE) was prepared according to the method of García-Iñiguez de Ciriano et al. (2009). Water extraction was performed in triplicate. Extracts were freeze-dried, previously freezing at −80 °C, and used, after rehydration with distilled water, for assaying antimicrobial activity. After freeze-drying, the yield in powder was 2.60 and 4.84 g from 100 g of fresh leaves of borage and Indian mustard, respectively. Lyophilized AEs were rehydrated adding distilled water till their complete dissolution (200 mg/mL for borage and 500 mg/mL for Indian mustard).

The strains used as indicators (sensitive to AEs) for the inhibition assays belonged to the culture collection of the Section of Hygiene, Department of Sciences for Health Promotion and Mother-Child Care "G. D'Alessandro" (University of Palermo, Italy) and account for some species commonly associated with foodborne diseases (Table 1). *Enterobacter* spp., *L. monocytogenes*, *S. aureus* and *Salmonella enterica* strains were subcultured in Brain Heart Infusion (BHI) (Oxoid, Milan, Italy) and incubated overnight at 37 °C.

2.2. Screening of the antibacterial activity and determination of the minimum inhibitory concentration

AEs from *B. officinalis* and *B. juncea* were tested for antibacterial activity applying the paper disc diffusion method reported by Militello, Settanni, Aleo, Mammina and Moschetti (2011). The indicator (sensitive) strains were tested at approximately 10^7 CFU/mL. Sterile water and streptomycin (10% w/v) were used as negative and positive control, respectively. Plates were incubated at 37 °C for 24 h and the inhibitory activity was evaluated as positive if a definite clear area was detected around the paper disc. Each test was performed in duplicate and repeated twice.

A completely randomised design was performed. Data of inhibitory activities were statistically analysed using a one-way ANOVA procedure. Differences between means were determined by Duncan's multiple-range test.

The antibacterial activity of the AEs was measured as minimum inhibitory concentration (MIC), which represents the most common expression of the antibacterial performances of a given active compound. MIC is defined as the lowest concentration of a substance inhibiting visible growth of test organisms (Karapinar & Aktug, 1987). AEs were serially diluted (2-fold) in water and all dilutions were tested against the most sensitive strains. Each test tube, containing 900 μL of broth medium and 100 μL of AE dilution, was then inoculated with approximately 10^6 CFU/mL of the sensitive strain. The bacterial growth was followed by optical density (OD), measured with a 6400 Spectrophotometer (Jenway Ltd., Felsted Dunmow, UK) at 600 nm. Sterile water alone was used as negative control.

2.3. Stability of the antibacterial activity and evaluation of cellular toxicity

In order to evaluate the stability over time, the screening of the antibacterial activity of the AEs, performed as reported above, was repeated after 1 year of storage at room temperature, 4 °C and −20 °C.

Cellular toxicity of the AEs (200 mg/mL) was assayed in presence of sheep erythrocytes after decimal serial dilution (till 0.2 mg/mL) of the extracts prepared in phosphate buffer saline (PBS) (Oxoid) following the methodology reported by Xian-Guo and Ursula (1994). PBS and tap water were used as negative and positive control, respectively. The tubes were incubated at 37 °C for 30 min and haemolysis was observed after centrifugation at 3000 × *g* for 5 min. Haemolysis was scored positive when the erythrocytes did not form a pellet after centrifugation.

2.4. In situ activity of plant AEs

In order to evaluate the *in situ* activity of *B. officinalis* and *B. juncea* AEs, they were added in three food model systems [meat broth (MB), fish broth (FB) and vegetable broth (VB)], prepared as described by Settanni et al. (2014) and sterilized by filtration through a 0.20 μm pore size filter (Sartorius, Aubagne Cedex,

Table 1
Inhibitory activity[a] of plant aqueous extracts against foodborne pathogenic bacteria.

Gram-positive strains	Isolation source	BJAE	BOAE	Gram-negative strains	Isolation source	BJAE	BOAE
L. monocytogenes 129	Human stools	7.5 d	0.0 d	*S.* Abony 50398	Human stools	0.0 f	0.0 e
L. monocytogenes 130	Human stools	8.0 d	0.0 d	*S.* Agona 50360	Human stools	0.0 f	0.0 e
L. monocytogenes 131	Human stools	8.5 d	0.0 d	*S.* Blockley 50314	Human stools	0.0 f	0.0 e
L. monocytogenes 132	Human stools	7.5 d	0.0 d	*S.* Bredeney 50374	Human stools	0.0 f	0.0 e
L. monocytogenes 133	Human stools	11.5 c	0.0 d	*S.* Derby 50399	Human stools	0.0 f	0.0 e
L. monocytogenes 134	Human stools	8.0 d	0.0 d	*S.* Enteritidis 50339	Food preparation	11.5 d	0.0 e
L. monocytogenes 135	Human stools	11.0 c	0.0 d	*S.* Enteritidis 50431	Molluscs	17.0 b	13.0 b
L. monocytogenes 136	Human stools	7.5 d	0.0 d	*S.* Hadar 50272	Eggs	0.0 f	0.0 e
L. monocytogenes 137	Human stools	8.5 d	0.0 d	*S.* Infantis 50270	Human stools	0.0 f	0.0 e
L. monocytogenes 138	Human stools	10.0 c	11.5 b	*S.* Muenchen 50393	Human stools	8.0 e	0.0 e
L. monocytogenes 139	Human stools	7.5 d	10.5 b	*S.* Napoli 50376	Human stools	10.5 d	0.0 e
L. monocytogenes 140	Human stools	0.0 e	0.0 d	*S.* Newport 50404	Human stools	0.0 f	0.0 e
L. monocytogenes 179	Salmon	0.0 e	0.0 d	*S.* Panama 50347	Human stools	0.0 f	0.0 e
L. monocytogenes 180	Ricotta cheese	0.0 e	0.0 d	*S.* Saintpaul 50415	Human stools	7.5 e	0.0 e
L. monocytogenes 182	Ricotta cheese	0.0 e	7.5 c	*S.* Thompson 50280	Chicken-pork meat	0.0 f	0.0 e
L. monocytogenes 184	Rice salad	8.0 d	8.0 c	*S.* Typhimurium 50414	Human stools	0.0 f	0.0 e
L. monocytogenes 185	Beef	0.0 e	8.5 c	*S.* Typhimurium 50432	Molluscs	0.0 f	0.0 e
L. monocytogenes 186	Mozzarella salad	7.5 d	7.5 c	*S.* Veneziana 50391	Human stools	12.5 c	11.5 c
L. monocytogenes 187	Roasted chicken	0.0 e	8.0 c	*Enterobacter* spp. 1435UTIN	Human stools	0.0 f	0.0 e
L. monocytogenes 188	Green salad	11.5 c	0.0 d	*Enterobacter* spp. 4UTIN	Human stools	0.0 f	13.5 b
L. monocytogenes 1BO	Chopped meat	11.0 c	0.0 d	*Enterobacter* spp. 5UTIN	Human stools	0.0 f	13.5 b
L. monocytogenes 2BO	Salami	10.5 c	0.0 d	*Enterobacter* spp. 7UTIN	Human stools	0.0 f	0.0 e
L. monocytogenes 3BO	Salami	7.5 d	0.0 d	*Enterobacter* spp. 8UTIN	Human stools	0.0 f	7.5 d
L. monocytogenes 4BO	Salami	11.0 c	0.0 d	*Enterobacter* spp. 9UTIN	Human stools	0.0 f	13.5 b
L. monocytogenes 5BO	Salami	0.0 e	0.0 d	*Enterobacter* spp. 10UTIN	Human stools	0.0 f	14.0 b
L. monocytogenes 6BO	Salami	8.0 d	0.0 d	*Enterobacter* spp. 12UTIN	Human stools	0.0 f	13.0 b
L. monocytogenes 7BO	Salami	0.0 e	0.0 d	*Enterobacter* spp. 17UTIN	Human stools	0.0 f	13.5 b
L. monocytogenes 8BO	Salami	8.5 d	8.0 c	*Enterobacter* spp. 19UTIN	Human stools	0.0 f	0.0 e
L. monocytogenes 11BO	Meat factory	7.5 d	0.0 d	*Enterobacter* spp. 20UTIN	Human stools	0.0 f	13.0 b
L. monocytogenes 12BO	Salami	0.0 e	8.5 c	*Enterobacter* spp. 28UTIN	Human stools	0.0 f	14.0 b
L. monocytogenes 13BO	Gorgonzola cheese	0.0 e	0.0 d	*Enterobacter* spp. 29UTIN	Human stools	0.0 f	16.5 a
L. monocytogenes 14BO	Gorgonzola cheese	0.0 e	7.5 c	*Enterobacter* spp. 30UTIN	Human stools	0.0 f	12.5 b
L. monocytogenes 15BO	Gorgonzola cheese	0.0 e	0.0 d	*Enterobacter* spp. 31UTIN	Human stools	0.0 f	14.0 b
L. monocytogenes 16BO	Gorgonzola cheese	0.0 e	0.0 d	*Enterobacter* spp. 33UTIN	Human stools	0.0 f	13.0 b
L. monocytogenes 17BO	Gorgonzola cheese	11.5 c	0.0 d	*Enterobacter* spp. 35UTIN	Human stools	0.0 f	0.0 e
L. monocytogenes 18BO	Gorgonzola cheese	0.0 e	0.0 d	*Enterobacter* spp. 36UTIN	Human stools	0.0 f	13.5 b
L. monocytogenes 19BO	Gorgonzola cheese	0.0 e	0.0 d	*E. hormaechei*[b] 1	Milk powder	17.5 b	10.5 c
L. monocytogenes 20BO	Gorgonzola cheese	0.0 e	0.0 d	*E. hormaechei*[b] 2	Milk powder	0.0 f	11.5 c
L. monocytogenes 21BO	Gorgonzola cheese	0.0 e	0.0 d	*E. hormaechei*[b] 6	Milk powder	0.0 f	13.5 b
L. monocytogenes 22BO	Taleggio cheese	8.0 d	11.0 b	*E. hormaechei*[b] 7	Milk powder	0.0 f	14.0 b
L. monocytogenes 23BO	Taleggio cheese	10.5 c	11.5 b	*E. hormaechei*[b] 8	Milk powder	16.5 b	11.0 c
L. monocytogenes 24BO	Taleggio cheese	11.5 c	0.0 d	*E. hormaechei*[b] 11	Milk powder	17.0 b	13.0 b
S. aureus C1/56340.0MSSA	Cheese	0.0 e	0.0 d	*E. hormaechei*[b] 13	Milk powder	17.5 b	8.0 d
S. aureus C4/6561.10.0MSSA	Raw milk	11.5 c	0.0 d	*E. hormaechei*[b] 19	Milk powder	17.5 b	8.5 d
S. aureus C6/51450.0MSSA	Cheese	11.0 c	0.0 d	*E. hormaechei*[b] 20	Milk powder	16.5 b	13.5 b
S. aureus C38/249.10.0MSSA	Cheese	7.5 d	0.0 d	*E. hormaechei*[b] 31	Milk powder	16.0 b	10.5 c
S. aureus C45/124250.0MSSA	Cheese	17.5 a	0.0 d	*E. cloacae* 24	Milk powder	17.5 b	11.5 c
S. aureus 1950.0MRSA	Human stools	0.0 e	0.0 d	*E. cloacae* 25	Milk powder	17.5 b	11.0 c
S. aureus TUM0.0MRS	Human stools	0.0 e	0.0 d	*E. cloacae* 13A	Multi-cereal cream	12.5 c	11.0 c
S. aureus 90.0MRS	Human stools	14.0 b	0.0 d	*E. cloacae* 62A	Freeze-dried chicken	17.0 b	11.5 c
S. aureus 140.0MRSA	Human stools	13.0 b	0.0 d	*E. cloacae* 32A	Milk flour	0.0 f	10.5 c
S. aureus 1890.0MRSA	Human stools	0.0 e	0.0 d	*E. cloacae* 43B1	Semolina	17.5 b	14.0 b
S. aureus 13130.0MRSA	Human stools	0.0 e	0.0 d	*E. sakazakii* 2B	Rice cream	16.5 b	0.0 e
S. aureus 13690.0MRSA	Human stools	0.0 e	0.0 d	*E. sakazakii* 23A	Green rice cream	20.5 a	13.5 b
S. aureus 5810.0MRSA	Human stools	17.5 a	0.0 d	*E. amnigenus* 70B3	Freeze-dried chicken	17.5 b	10.5 c
S. aureus 3400.0MRSA	Human stools	16.5 a	7.5 c	*E. amnigenus* 60A2	Freeze-dried lamb	20.0 a	13.5 b
S. aureus 4ADI MRSA	Human stools	0.0 e	8.0 c				
S. aureus 7ADI MSSA	Human stools	0.0 e	0.0 d				
S. aureus 14LU MRSA	Human stools	13.5 b	7.0 c				
S. aureus 16 MSSA	Human stools	13.0 b	7.5 c				
S. aureus 19 MRSA	Human stools	0.0 e	11.0 b				
S. aureus 20ADI MRSA	Human stools	7.0 d	0.0 d				
S. aureus 21ADI MRSA	Human stools	7.5 d	0.0 d				
S. aureus 62 MRSA	Human stools	17.0 a	11.5 b				
S. aureus 68 MRSA	Human stools	14.0 b	13.5 a				
S. aureus 106 MRSA	Human stools	17.5 a	13.0 a				
S. aureus 109 MRSA	Human stools	0.0 e	0.0 d				
S. aureus 156 MRSA	Human stools	13.5 b	8.0 c				
S. aureus 168 MRSA	Human stools	0.0 e	7.5 c				
S. aureus 206 MSSA	Human stools	0.0 e	7.5 c				
S. aureus 473 MRSA	Human stools	13.0 b	10.5 b				
S. aureus 493 MRSA	Human stools	13.5 b	8.0 c				
S. aureus 637 MRSA	Human stools	13.5 b	7.5 c				

(*continued on next page*)

Table 1 (*continued*)

Gram-positive strains	Isolation source	BJAE	BOAE	Gram-negative strains	Isolation source	BJAE	BOAE
S. aureus 734 MSSA	Human stools	0.0 e	0.0 d				
S. aureus 735 MSSA	Human stools	0.0 e	0.0 d				
S. aureus 750 MSSA	Human stools	0.0 e	0.0 d				
S. aureus E36GI MRSA	Human stools	17.5 a	11.0 b				

Abbreviations: BJAE, Brassica juncea aqueous extract; BOAE, Borago officinalis aqueous extract, MSSA, methicillin susceptible S. aureus; MRSA, methicillin resistant S. aureus.
[a] Inhibitory activity of plant aqueous extracts is indicated by the width of the inhibition zone (mm) around the paper disc. Results indicate mean values of four replicates (carried out in duplicate and repeated twice). Data within a column followed by the same letter are not significantly different according to Duncan's multiple range test.
[b] *E. hormaechei* subsp. *steigerwaltii*.

France). The tests were carried out using one of the most sensitive and one non sensitive strain to the AEs within each bacterial species. Each strain was grown overnight in BHI broth; cells were harvested by centrifugation at 5000 × *g* for 5 min, washed with Ringer's solution (Sigma–Aldrich, Milan, Italy), and, to standardise bacterial inocula, resuspended in the same solution to an optical density at 600 nm of 1.00, corresponding to approximately 10^9 CFU/mL, as measured by Spectrophotometer. The final inoculation of the strains was at about 10^4 CFU/mL to mimic a massive contamination. The AEs were filter sterilized and singly added to each food model system at two final concentrations, those corresponding to the MIC estimated *in vitro* and 10-fold higher. Tests in BHI were carried out for comparison. Incubation was at 25 °C for 24 h to simulate a prolonged interruption of the cold chain. Plate counts were performed to enumerate the surviving cells. The broths (1 mL) were subjected to the serial decimal dilution in Ringer's solution and the cell suspensions were spread plated (0.1 mL) onto BHI agar and incubated at 37 °C for 24 h. Tests were carried out in duplicate.

2.5. Inflence of AEs on the volatile organic compound composition of different food models

The influence of *B. officinalis* and *B. juncea* AEs on the flavour of foods, was estimated by analyzing the volatile organic compounds (VOCs) of the three food model systems reported above (MB, FB and VB). VOCs were analysed before and after addition of AEs. Both AEs were singly tested at a final concentration corresponding to the lowest concentration that caused the *in situ* inhibition of the most sensitive strains in each food model system. VOCs within VB, MB and FB were identified using the solid phase micro extraction (SPME) isolation technique in 5 mL volume vials and analysed by gas chromatography/mass spectrometry as reported by Alfonzo et al. (2013). Individual peaks were identified by comparing their retention indices to those of control samples and by comparing their mass spectra with those within the NIST/EPA/NIH Mass Spectral Library database (Version 2.0d, build 2005). Volatile compounds were expressed as relative peak areas (peak area of each compound/total area) × 100. All solvents and reagents were purchased from WWR International (Milan, Italy). Chemical and physical tests were performed in triplicate, with the results expressed as mean ± standard deviation.

2.6. Sniffing assay

A sniffing test, carried out as described by Klein, Maillard, Thierry, and Lortal (2001), was performed on the food model systems added with AEs as reported above for VOC analysis (without bacterial inocula) and incubated for 24 h at 25 °C. The sensory panel was composed of eight assessors (four females and four males, 27–43 years old) which were trained with the raw ingredients of each food model (homogenized and mixed together). The panellists were asked to evaluate the differences among the broths with and without addition of the AEs at the final concentrations active *in situ*. The broths (15 mL) were put in test tubes and presented to the assessors in plain view.

3. Results and discussion

3.1. Antibacterial activity of plant aqueous extracts

The antibacterial activity of the AEs analysed in this study is shown in Table 1. The spectrum of inhibition was evaluated against *Enterobacter* spp. (38 strains), *L. monocytogenes* (42 strains), *S. aureus* (35 strains) and *S. enterica* (18 strains) which are bacterial species reported to be responsible for human diseases and are commonly associated with the consumption of contaminated food items (Crum-Cianflone, 2008; Healy et al., 2010; Swaminathan & Gerner-Smidt, 2007; Wilson et al., 2000). Both AEs showed a negligible inhibition of *L. monocytogenes* strains and, with a few exceptions, *salmonellae*, but the activity towards staphylococci and enterobacteria was found to be interesting. Apart some *Enterobacter* isolates from human stool samples and some strains of *Enterobacter hormaechei* subsp. *steigerwaltii*, the activity of AE from Indian mustard was generally higher than that registered for borage in terms of both number of strains inhibited and width of the inhibition zone. The first AE showed a width of the inhibition halo in the range 15–18 mm or higher for several strains, including *S. aureus* and *E. hormaechei* subsp. *steigerwaltii*, *Enterobacter cloacae*, *Enterobacter sakazakii* and *Enterobacter amnigenus*. *B. officinalis* AE showed a clear area in the range 15–18 mm only in one case, against *Enterobacter* spp. 29UTIN. Although the susceptibility to the treatments was proved to be strain-dependent, several strains were inhibited by *B. officinalis* and *B. juncea* AEs (57 and 67 strains, respectively), showing large inhibitory spectra of these extracts and their suitability for practical use in preservation.

Plants of Boraginaceae family have already been tested against *S. aureus*: *Echium amoenum* showed antagonistic properties (Abolhassani, 2004), while *Cordia latifolia* and *Onosma bracteatum* did not possess inhibitory power (Ahmad et al., 1998). The last two species were not effective against *S.* Typhimurium, the only *S. enterica* serovar tested by Ahmad et al. (1998). No previous studies had been carried out on the effect of *B. officinalis* AE on *L. monocytogenes*, several serovars of *S. enterica* and different species of *Enterobacter* genus.

Regarding the antibacterial potential of *B. juncea*, no activity was registered *vs. S. aureus* and *S.* Typhimurium by Ahmad et al. (1998). No further information is available in literature on its behaviour against the other species used in the present study.

L. monocytogenes 138 and 23BO, methicillin resistant *S. aureus* (MRSA) 68 and MRSA 106, *S.* Enteritidis 50431, *E. sakazakii* 23A and *E. amnigenus* 60A2 were among the most sensitive strains of each species, in terms of width of the inhibitory halo determined by both AEs, and for this reason they were chosen for the calculation of the MIC. The two AEs showed different inhibitory efficacies. Except against *L. monocytogenes* strains for which the MIC was 20 mg/mL, borage AE showed the same MIC of 10 mg/mL against

the majority of the most sensitive strains. The MIC evaluated for the Indian mustard AE was different for each strain: 25 mg/mL *vs L. monocytogenes* 138 and 23BO, 12.5 mg/mL *vs S. aureus* 68 MRSA, 6.2 mg/mL *vs S. aureus* 106 MRSA and *S.* Enteritidis 50431, and 3.1 mg/mL *vs E. sakazakii* 23A and *E. amnigenus* 60A2. These differences may be important in food applications; the first aspect to be considered before the inclusion of a given plant extract to control the bacterial growth in foods is its effect on the organoleptic profile of the final products. Naturally derived preservatives can alter the taste of foods or exceed acceptable flavour thresholds (Hsieh, Mau, & Huang, 2001; Nazer, Kobilinsky, Tholozana, & Dubois-Brissonneta, 2005). For this reason, low concentrations of active compounds limit the risk of changes in the sensory features of the treated foods.

3.2. Stability and cytotoxicity of plant aqueous extracts

The stability of the AEs over time was also evaluated: no AE retained its inhibitory power against the seven most sensitive strains after 1-year storage in different thermal conditions.

Haemolysis of sheep erythrocytes was negative at any dilution of both AEs and in presence of PBS. On the contrary, tap water determined a clear erythrocyte haemolysis, demonstrating that the plant extracts did not contain cytotoxic compounds. Based on this

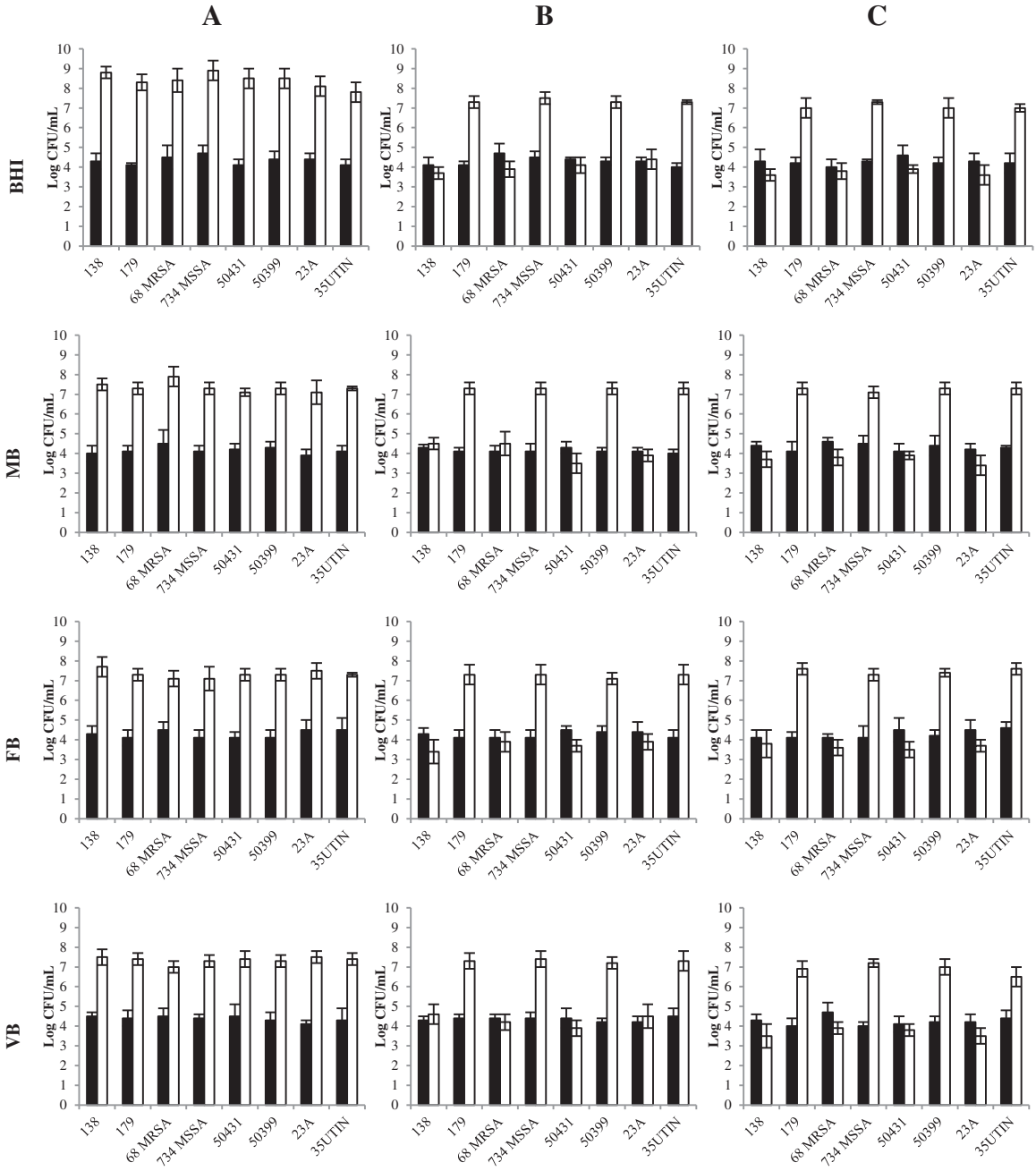

Fig. 1. *In situ* activity of aqueous extracts. A, control trials; B, trials added with *B. juncea* AE; C, trials added with *B. officinalis* AE. Abbreviations: BHI, brain heart infusion; MB, meat broth; FB, fish broth; VB, vegetable broth. Strains: *L. monocytogenes* 138, *L. monocytogenes* 179, *S. aureus* 68MRSA, *S. aureus* 734 MSSA, *S.* Enteritidis 50431, *S.* Derby 50399, *E. sakazakii* 23A, *Enterobacter* spp. 35UTIN. Vertical bars represent standard deviation of the mean.

characteristic, *B. officinalis* and *B. juncea* AEs may have different *in vivo* applications, e.g. in antiseptic or disinfectant formulations, in chemotherapy, in food production, etc.

3.3. In situ tests

The effect of both *B. officinalis* and *B. juncea* AEs added at the final concentrations that showed inhibition *in vitro* was not confirmed *in situ*, since the growth of all strains in presence of the active extracts was comparable with that estimated in their absence (results not shown). These observations are not surprising because the activity of the inhibitory substances in foods might be negatively influenced by various factors, such as binding of the active compounds to food components or food additives, inactivation by food inhibitors, changes in solubility and charge and changes in the cell envelope of the target bacteria. However, when the AEs were added at final concentrations 10-fold higher that those active *in vitro* (100 and 31 mg/mL for borage and Indian mustard, respectively), their inhibitory effect was clearly registered (Fig. 1). All sensitive strains behaved similarly to the non-sensitive strains in absence of AEs, in all food model systems, that supported the growth of all test bacteria at high levels, although almost 1 Log lower than growth detected in the synthetic medium. In presence of both AEs the growth of the sensitive strains did not occur, but they were still viable, at almost the same levels of inoculation, after 24 h contact with the plant extracts. Thus, the *in situ* mode of action of *B. officinalis* and *B. juncea* AEs was proved to be bacteriostatic.

Table 2
Analysis of volatile organic compounds emitted from food model systems added with plant aqueous extracts.

Chemical compounds[a]	Samples								
	MB			FB			VB		
	Control	+BJAE	+BOAE	Control	+BJAE	+BOAE	Control	+BJAE	+BOAE
Alcohols									
1-Pentanol	4.1 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	n.d.	n.d.	n.d.	1.6 ± 0.1	1.3 ± 0.1	1.0 ± 0.0
2,3-Butanediol	n.d.	3.2 ± 0.1	0.5 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-Methyl-2-hexanol	1.7 ± 0.1	3.1 ± 0.1	1.6 ± 0.2	n.d.	n.d.	n.d.	0.3 ± 0.0	n.d.	n.d.
1-Hexanol	1.9 ± 0.0	2.2 ± 0.0	2.1 ± 0.1	3.4 ± 0.1	2.9 ± 0.1	4.1 ± 0.1	41.1 ± 0.6	44.6 ± 1.3	50.6 ± 1.4
3-Hexen-1-ol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	83.1 ± 10.3	88.3 ± 8.2	88.4 ± 5.2
2-Hexen-1-ol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	24.1 ± 1.6	23.1 ± 2.6	24.5 ± 4.4
1-Octen-3-ol	16.5 ± 2.0	17.1 ± 0.2	18.0 ± 0.2	6.9 ± 1.4	3.5 ± 1.2	9.5 ± 0.1	3.8 ± 1.7	1.9 ± 0.9	4.8 ± 1.2
1-Hepanol	7.4 ± 0.1	7.7 ± 0.1	9.7 ± 0.9	2.3 ± 0.7	1.5 ± 0.2	2.5 ± 0.2	2.6 ± 0.6	2.0 ± 0.7	2.5 ± 0.3
1-Octanol	17.2 ± 0.2	19.8 ± 0.2	23.4 ± 0.2	3.5 ± 0.2	2.2 ± 0.2	3.5 ± 0.2	9.5 ± 1.1	9.0 ± 1.0	10.4 ± 1.4
4-Terpineol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	26.7 ± 1.3	28.2 ± 1.5	29.1 ± 2.0
2-Nonen-1-ol	1.4 ± 0.2	1.5 ± 0.2	1.1 ± 0.2	n.d.	n.d.	n.d.	n.d.	1.4 ± 0.3	n.d
α-Terpineol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	55.1 ± 2.7	55.4 ± 2.5	58.2 ± 2.0
1-Dodecanol	n.d.	n.d.	2.9 ± 0.0	n.d.	n.d.	n.d.	2.1 ± 0.0	3.3 ± 0.1	1.9 ± 0.1
1,4-Butanediol	2.2 ± 0.0	1.9 ± 0.0	n.d.	n.d.	1.9 ± 0.1	3.2 ± 0.1	4.1 ± 0.7	6.9 ± 0.1	3.2 ± 0.1
Aldehydes									
Hexanal	311.9 ± 41.0	340.6 ± 35.2	351.2 ± 21.9	8.4 ± 2.1	3.4 ± 0.2	13.0 ± 1.1	53.7 ± 2.6	52.1 ± 3.2	53.5 ± 5.2
Heptanal	53.0 ± 1.49	52.7 ± 5.1	60.9 ± 6.2	n.d.	n.d.	n.d.	17.1 ± 1.2	29.9 ± 1.2	15.1 ± 1.1
2-Hexenal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	281.4 ± 21.4	292.5 ± 19.8	278.5 ± 28.1
Octanal	63.8 ± 4.5	63.6 ± 6.3	70.2 ± 5.5	11.0 ± 0.9	4.1 ± 0.5	9.6 ± 1.8	64.4 ± 5.3	66.9 ± 5.0	66.8 ± 6.7
2-Heptenal	2.8 ± 0.1	3.1 ± 0.2	6.6 ± 0.3	n.d.	n.d.	n.d.	5.6 ± 1.1	5.0 ± 0.2	5.3 ± 1.3
2-Pentenal	2.3 ± 0.2	1.9 ± 0.1	2.9 ± 0.1	n.d.	n.d.	n.d.	2.2 ± 0.4	2.0 ± 0.3	2.1 ± 0.7
Nonanal	42.4 ± 4.4	60.0 ± 4.9	32.9 ± 2.2	48.6 ± 5.9	45.5 ± 1.2	46.8 ± 4.2	21.2 ± 3.1	23.0 ± 2.1	18.0 ± 1.4
2,4-Hexadienal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.0 ± 1.1	11.3 ± 1.1	7.9 ± 1.3
2-Octenal	29.8 ± 6.4	36.9 ± 4.1	66.3 ± 6.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Furfural	n.d.	1.3 ± 0.1	3.0 ± 0.2	12.1 ± 2.1	5.9 ± 1.8	11.7 ± 2.3	6.8 ± 0.9	10.7 ± 1.3	5.6 ± 1.4
2,4-Heptadienal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.7 ± 0.2	2.3 ± 1.1	2.7 ± 1.0
Decanal	6.4 ± 0.5	17.5 ± 0.8	7.6 ± 0.8	22.1 ± 2.1	9.9 ± 2.0	12.4 ± 3.0	4.3 ± 0.9	5.3 ± 1.8	8.4 ± 1.0
Benzaldehydes	8.8 ± 0.9	13.4 ± 2.5	7.9 ± 0.5	8.1 ± 1.1	5.4 ± 0.9	7.7 ± 1.1	80.3 ± 6.4	85.0 ± 4.1	85.1 ± 5.8
2-Nonenal	20.4 ± 1.9	20.4 ± 1.5	37.5 ± 2.6	n.d.	n.d.	n.d.	13.6 ± 1.6	12.1 ± 1.2	13.1 ± 2.3
Undecanal	n.d.	10.2 ± 1.0	n.d.	1.8 ± 0.3	n.d.	n.d.	n.d.	n.d.	n.d.
2-Decenal	49.8 ± 7.4	51.2 ± 8.7	91.8 ± 12.3	2.1 ± 0.8	n.d.	n.d.	8.3 ± 1.3	8.6 ± 1.5	8.8 ± 1.1
2-Hydroxy-Benzaldehyde	n.d.	n.d.	n.d.	15.0 ± 1.1	16.7 ± 0.4	14.9 ± 1.2	n.d.	n.d.	n.d.
2,4-Nonadienal	10.1 ± 1.0	10.8 ± 0.9	12.6 ± 1.3	n.d.	n.d.	n.d.	4.9 ± 0.4	2.9 ± 1.3	2.9 ± 1.6
2-Undecenal	39.4 ± 2.3	38.5 ± 4.1	69.5 ± 5.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,4-dimethyil-Benzaldehyde	4.8 ± 0.9	6.8 ± 1.4	9.9 ± 1.2	95.8 ± 8.2	38.2 ± 4.5	82.7 ± 3.2	14.2 ± 2.2	20.4 ± 3.1	19.0 ± 1.6
2,4-Decadienal	1.7 ± 0.2	2.2 ± 0.3	2.8 ± 0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isophthalaldehyde	0.9 ± 0.1	0.7 ± 0.4	0.9 ± 0.5	1.4 ± 0.2	1.4 ± 0.2	1.7 ± 0.3	2.3 ± 0.4	1.7 ± 0.8	2.0 ± 0.7
Esters									
Butyrolactone	8.3 ± 1.8	4.6 ± 0.4	3.5 ± 0.4	2.43 ± 0.3	2.6 ± 0.2	2.3 ± 1.1	16.6 ± 1.9	16.3 ± 2.1	15.9 ± 1.0
Hydrocarbons									
6-Methyl-1-octene	1.5 ± 0.4	1.8 ± 0.6	9.9 ± 2.4	15.4 ± 1.6	1.4 ± 0.4	10.2 ± 1.4	5.2 ± 1.9	5.8 ± 0.2	4.8 ± 1.2
Ketones									
2,3-Octanedione	6.2 ± 0.6	6.1 ± 1.4	9.5 ± 2.1	n.d.	n.d.	n.d.	1.9 ± 0.3	3.0 ± 1.2	2.8 ± 0.5
3-Octen-2one	1.5 ± 0.2	1.8 ± 0.3	2.1 ± 0.2	n.d.	n.d.	n.d.	5.2 ± 1.7	6.8 ± 0.8	5.9 ± 0.9
2-Nonanone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.7 ± 0.9	2.7 ± 0.9	2.6 ± 0.8
β-Damascenone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.5 ± 0.8	5.8 ± 0.7	6.5 ± 1.8
Phenols									
Phenol	1.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	5.0 ± 0.5	2.7 ± 0.3	5.5 ± 1.0	2.8 ± 0.4	3.1 ± 0.6	2.9 ± 0.7

Abbreviations: MB, meat broth; FB, fish broth; VB, vegetable broth; BJAE, Brassica juncea aqueous extract; BOAE, Borago officinalis aqueous extract, Results indicate mean values of three measurements and are expressed (in mg/kg) as 4-methyl-2-pentanone. n.d., not detected.

[a] The chemicals are shown following their retention time within each class.

3.4. Influence of AEs on the flavour of foods

VOC generation, established from chromatographic analysis, is reported in Table 2. Based on the results of the *in situ* activity determination, *B. juncea* AE was added at a final concentration of 31 mg/mL, while *B. officinalis* AE at 100 mg/mL in each food model system. Within the headspace of the broths without addition of AEs (control broths), 29 compounds were identified for MB (8 alcohols, 16 aldehydes, 1 ester, 1 hydrocarbon, 2 ketones and phenol), 18 compounds for FB (4 alcohols, 11 aldehydes, 1 ester, 1 hydrocarbon and phenol) and 36 compounds for VB (12 alcohols, 17 aldehydes, 1 ester, 1 hydrocarbon, 4 ketones and phenol). After the addition of *B. juncea* AE, 32 compounds were identified for MB (9 alcohols, 18 aldehydes, 1 ester, 1 hydrocarbon, 2 ketones and phenol), 17 compounds for FB (5 alcohols, 9 aldehydes, 1 ester, 1 hydrocarbon and phenol) and 36 compounds for VB (the same aldehydes, ester, hydrocarbon and phenol, and almost the same alcohols detected for control broth with the exception of 2-nonen-1-ol in place of 3-methyl-2-hexanol). After the addition of *B. officinalis* AE, 31 compounds were identified for MB (9 alcohols, 17 aldehydes, 1 ester, 1 hydrocarbon, 2 ketones and phenol), 17 compounds for FB (5 alcohols, 9 aldehydes, 1 ester, 1 hydrocarbon and phenol) and the same 36 compounds detected in presence of *B. juncea* AE for VB.

Aldehydes were the chemicals detected at the highest concentrations in all broths. Hexanal, whose concentration was also high in control broths, was the VOC present in the highest quantity in all MBs, while 2-hexenal in all VBs. 2,4-dimethyl-benzaldehyde represented the major VOC in FBs, but the concentration estimated after the addition of *B. juncea* AE was consistently lower than those measured for control FB and FB after the addition of *B. officinalis* AE.

B. juncea AE determined the presence of 2,3-butanediol, furfural and undecanal, while *B. officinalis* 2,3-butanediol, 1-dodecanol and furfural in addition to the VOCs identified for control MB. After the addition of *B. juncea* and *B. officinalis* AEs, the VOC fraction of FB contained 1,4-butanediol, but disappeared undecenal and 2-decenal. Regarding the chemicals detected before and after addition of both AEs, several differences were observed in terms of concentration. Thus, the addition of *B. juncea* and *B. officinalis* AEs affected the flavour of the food models used in this study.

3.5. Analysis of general odour

The eight assessors judged all food models added with *B. officinalis* AE as being characterised by the same general odour of the broths not added. Two assessors recognized FB added with *B. juncea* AE as different from FB without addition, and only one panellist recognised also MB and VB added with *B. juncea* AE as different from the corresponding broths not added.

4. Conclusions

The aqueous extracts of *B. officinalis* and *B. juncea* showed inhibitory activity at different extent especially towards staphylococci and enterobacteria. In terms of number of sensitive strains and MICs, *B. juncea* extract showed higher inhibitory activity than *B. officinalis*. Although the inhibitory power was lost during storage, the extracts could have interesting application in short-time preservation of different products against bacterial pathogens. Furthermore, the absence of cytotoxicity make them suitable as natural antibacterial substances for food and/or feed. The *in situ* tests demonstrated that both AEs were active at a concentration 10-fold higher than that showing definite efficacy *in vitro*. The addition of both AEs at concentrations biological active *in situ* determined a modification of the VOCs of the food model systems. However, the sniffing assay found only *B. juncea* AE impacting consistently the flavour of the food models. Although the broths used in this study were model systems different from real food matrices, our data can be used to further evaluate the role of plant AEs in foods. For this reason, we concluded that *B. officinalis* AE finds application as biopreservative and will be better investigated in several food matrices.

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