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INFLUENCE OF THE HERB EXTRACT ON INHIBITION OF BEEF MEAT SPOILAGE - POTENTIAL SOURCE OF NATURAL PRESERVATIVE

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ABSTRACT: Raw beef can be contaminated by microorganisms and support the growth of pathogens, and may lead to serious food-borne diseases. In many cases, plant extracts exibit antimicrobial and antioxidant activity. We investigated the inhibitory activity of 2.5% ethanol extract of Serbian herb *Kitaibelia vitifolia* against ATCC strains: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus hauseri*, *Proteus mirabilis*, *Bacillus subtilis* and fungi *Candida albicans* and *Aspergillus niger*, in a lean beef meat. We prepared samples (48 pieces per 25 g), according the sterility demands, from one piece of beef (2 kg). We organized 3 experimental group: samples from first (I - control) group are non-treated; II - samples immersed in sterile water and III - samples immersed in above mentioned herb extract. Analysis was carried out during storage at 4 ^{0}C and 25 ^{0}C (0. d, after 2, 4 and 7 d). Longest sustainability shows samples from experimental group III, on both temperature of storage, determined by method for proving spoilage (Nessler). Antimicrobial activity evaluated by their minimum inhibitory concentrations (MIC). The above mentioned extract showed strong inhibitory activity against *E.coli* (7.820 mg/mL), *S. aureus*, *P. mirabilis and K. pneumoniae* (15.625 mg/mL). Moderate sensibility on applyed herb extract shown *P. hauseri* (31.250 mg/mL) and minimum of inhibitory activity against *B. subtilis* (62.500 mg/mL). Among fungi, *A.niger* is very susceptible (7.820 mg/mL), unlike the *C. albicans* (62.500 mg/mL). This extract may be further investigated as a natural preservative to the food industry.
Key words: *beef, spoilage, antimicrobial activity, herb extract, Kitaibelia vitifolia*

INTRODUCTION

Diseases caused by eating food contaminated with pathogens around the world have strong economic, as well as the impact on public health (Gandhi and Chikindas, 2007). Microorganisms can adapt to survive and grow in a wide level of ambient conditions and the variety of raw and processed foods, including milk and dairy products, various kinds of meat and meat products and fresh products. Failure of food includes physical and chemical changes, oxidation, discoloration, or the occurrence of unpleasant taste and odor, as a result of the growth of microorganisms and their metabolic products (Gram et al., 2002). Spoilage of chilled meat is mainly caused by *Pseudomonas species*, which cause taste and odor, discoloration, production of gas and mucus (Oussalah et al., 2006a). Many pathogenic microorganisms including *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Campylobacter jejuni, Candida spp., Zygosaccharomyces spp., Fusarium spp., Aspergillus spp., Rhizopus spp., Penicillium spp.,* and *Salmonella spp.* have been identified as causes of "food-borne" disease or spoilage of foods (Betts et al., 1999; Solomakos et al., 2008).
Davidson et al. (2000) reported that the natural herb extracts are known to have huge antimicrobial activities and listed as Generally Recognized As Safe (GRAS). Many natural herbal extracts contain mainly phenolic compounds, which are potent antioxidants (Wong et al., 1995), and occur only in materials of plant origin. Resistance to antibiotics has become a subject of global concern and interest. In recent years there is a growing number of cases of multiple resistance in terms of pathogenic microorganisms in the human population, mainly

due to reckless commercial use of antibacterial drugs widely used in the treatment of infectious diseases (Westh et al., 2004).
This fact reinforces scientists that are exploring new antimicrobial substances originating from various sources, such as medicinal plants. This investigation is continuing by screening a large number of plant families (Parekh and Sumitra, 2007). Ghasemi et al. (2010) determined the antimicrobial activity of the extracts of eight plant species which are endemic in Iran, by agar disc diffusion and serial dilution assays. Most of the extracts showed a relatively high antimicrobial activity against all the tested bacteria and fungi. Some studies show that plant extracts useful for reducing pathogen contamination in meat (Mytle et al., 2006; Ahn et al. 2007), while others report that the very low or no antimicrobial activity against *L. monocytogenes* or *Salmonella* when they are essential plant oils applied to the beef or chicken (Uhart et al., 2006; Firouzi et al., 2007).The aim of our study is to determine antimicrobial influence of ethanol extract of Serbian herb *Kitaibellia vitifolia* on delaying of the lean beef meat spoilage, at usual temperatures of storage (4 ^{0}C and 25 ^{0}C).

MATERIAL AND METHODS

Plant material

Kitaibelia vitifolia is an imposing & undemanding Mallow from ex-Yugoslavia. Above-ground part of the test plant was collected in Central Serbia, in May 2009, at the flowering stage. Taxonomy of plant was identified and the voucher specimen was deposited at the Department of Botany, Faculty of Biology, University of Belgrade (16350 BEOU, Lakušić Dmitar).

Preparation of extract

Herb samples (10.0 g) were extracted by 96 % ethanol as a solvent. The extraction process was carried out using an ultrasonic bath (Brason and Smith-Kline Company, B-220) at room temperature for 1 hour. After filtration, 5 mL of the liquid extract was used for extraction yield determination. The solvent was removed by a rotary evaporator (Devarot, Elektromedicina, Ljubljana) under vacuum, and was dried at 60 ^{0}C to constant weight. The dried extracts were stored in glass bottles at 4 ^{0}C to prevent oxidative damage until analysis.

Spectrophotometric measurements

Spectrophotometric measurements were performed using a UV-VIS spectrophotometer MA9523-SPEKOL 211 (ISKRA, Horjul, Slovenia).

Samples

Samples of lean beef meat (48 pieces per 25 g) prepared from one piece of beef (2 kg). To simulate the usual way of meat preparing, samples split up and macerated using a knife (scalpel), according the sterility demands, in laminar flow biological safety cabinet Iskra IBK 1H2. Samples were measured on a technical scale (KERN EW 150-3M, Kern/Sohn GmbH, D-72 336 Balingen, Germany, M-2-60, CE 07, ISO 9001). We organized 2 experimental group (EG): twenty four samples from control (C) group are immersed in sterile buffered peptone water (8.5 g NaCl, M = 58.44 g/mol, JUS H. G2. 081, 9431, pro analysi, „ZORKA Pharma“ a.d. Šabac and 1 g of Pepton - Pepton-1, Lot 404001, Institute for virology, vaccines and serums, Vojvode Stepe 458, 11221 Belgrade, Serbia) in 1000 mL distilled water) and prepared solution sterilised by autoclaving, at 121 ^{0}C during 20 minutes; EG I - twenty four samples immersed in above mentioned 2.5 % herb extract (pH = 4.60). Duration of immersing the samples in the study groups was 5 minutes. Excess solution from samples of both (C and EG I) group was removed by placing the dipped pieces onto a sterilized paper towel before transferring them to sterile flask for incubation. Qualitative physic-chemical analysis was carried out during storage at 4 ^{0}C and 25 ^{0}C (0 d to 7 d) and micro dilution method performed with watery meat extract on the 7 d of the experiment.

Antimicrobial activity

Minimum inhibitory concentrations (MIC) of the sausage extract and against the test bacteria were determined using a micro dilution method in 96 multi-well micro-titer plates (Satyajit et al., 2007). All tests were performed in Muller-Hinton broth (MHB) with the exception of yeast, in which case Sabouraud dextrose broth was used. A total of 100 µL stock solution of sausage extract (in ethanol, 200 µL/mL) was pipetted into the first row of the plate. Fifty µL of Mueller-Hinton or Sabouraud dextrose broth (supplemented with Tween 80 at a final concentration of 0.5% (v/v) for analysis of sausage extract) was added to the other wells. Fifty µL from the first test wells was pipetted into the second well of each microtiter line, and then 50 µL of scalar dilution was transferred from the second to the twelfth well. Ten µL of resazurin indicator solution (prepared by dissolution of a 270-mg tablet in 40 mL of sterile distilled water) and 30 µL of nutrient broth were added to each well. Finally, 10 µL of bacterial suspension (10^6 CFU/mL) and yeast spore suspension (3×10^4 CFU/mL) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked. The standard antibiotic Amracin was used to control the sensitivity of the tested bacteria, whereas Ketoconazole was used as the control against the tested yeast. Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and prepared in triplicate, and then they were placed in an incubator at 37 ^{0}C for 24^h for the bacteria and at 28 ^{0}C for 48^h for the yeast. Color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value.

Test microorganisms

The antimicrobial activity of the herb extract was tested against the following bacteria: *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 13315, *Proteus mirabilis* ATCC 14153, *Bacillus subtilis* ATCC 6633, and fungi: *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404, in meat extracts prepared from examined lean beef meat pieces, after 8 d of storage. The fungi were cultured on potato-glucose agar for 7 d at room temperature of 20 ^{0}C under alternating light/dark conditions. Then they were cultured on a new potato-glucose substrate for another 7 d. The culturing procedure was performed four times, after which pure cultures required for determination were obtained. The identification of the test microorganisms was confirmed by the Laboratory of Mycology, Department of Microbiology, Institute Torlak, Belgrade, Serbia.

Determination of microbial spoilage

To assess the freshness and quality of examined meat sample during various ways of storage was determined the bound ammonia (easily hydrolyzable nitrogen) with *Nessler* reagent (tetraiodomercurate bipotasic solution in potassium hydroxide), which prepared with 5 g potassium iodide dissolved in 5 cm^3 of hot water in an Erlenmeyer flask. Hot saturated solution of mercuric chloride added until the precipitate formed is no longer dissolved. After cooling the solution separate, decant a 100 cm^3 volumetric flask. Add 15 g potassium hydroxide dissolved in 30 cm^3 water and bring to volume with water. Add 0.5 cm^3 saturated solution of mercuric chloride, allow to make the solution above the precipitate and separated by decantation, pass in a clean dark bottle and kept from light.

The reaction of the meat extract with Nessler's reagent may lead to the formation of turbidity and change of color and indicate slight decomposition. If a precipitate forms, the decomposition is advanced.

RESULTS AND DISCUSSION

The results on antimicrobial effects of herb extract obtained by the micro dilution method are shown in Table 1. As it can be seen from the results, MICs were determined for 8 indicator strains, and revealed that ethanol herb extract of *K. vitifolia* showed inhibitory effects tithing the concentration range of 7.820 µg/mL to 62.500 µg/mL.

Above mentioned herb extract was exhibited strongest inhibitory activity against *E.coli* (7.820 µg/mL), *S. aureus*, *P. mirabilis and K. pneumoniae* (15.625 µg/mL). Moderate sensibility on applyed herb extract shown *P. vulgaris* (31.250 µg/mL). Lowest inhibitory activity herb extract exhibited against *B. subtilis* (62.500 µg/mL). Among fungi, *A. niger* is very susceptible (7.820 µg/mL), unlike the *C. albicans* (62.500 µg/mL). These results are very similar like results were reported by Kurcubic et al. (2011), obtained in examination of the same influence in fermented dry Sremska sausages, but different of data presented by Mašković et al. (2011) for determination of MIC of the ethanol extract of *K. vitifolia* and standard drugs for same 8 indicator strains, *in vitro*.

Our opinion is that the reasons for this differences arising form interaction between microbes and constituents of meat or some other microorganisms from meat, which alter their ability for defense responses, or protect some examined strains. Generally, the efficiency of most antimicrobial properties of natural supplements can be reduced by the action of certain food components (Glass and Johnson, 2004). Cutter (2000) suggested that the use of herb extracts may afford some reductions of pathogens on beef surfaces, because the antimicrobial activity may be diminished in ground beef by adipose components.

Table 1. Minimum inhibitory concentrations (MIC) of the ethanolic extract of *K. vitifolia* for microbial strains in examined samples of meat extract

		MIC (µg/mL)		
Microbial strains	***ATCC number***	***Meat extract of EG I***	***Amracin***	***Ketoconazole***
		Bacteria		
Staphylococcus aureus	*25923*	15.625	0.970	/
Klebsiella pneumoniae	*13883*	15.625	0.490	/
Escherichia coli	*25922*	7.820	0.970	/
Proteus vulgaris	*13315*	31.250	0.490	/
Proteus mirabilis	*14153*	15.625	0.490	/
Bacillus subtilis	*6633*	62.500	0.240	/
		Funghi		
Candida albicans	*10231*	62.500	/	1.950
Aspergillus niger	*16404*	7.820	/	0.970

Table 2. The dynamic of investigated qualitative physic-chemical changes in stored macerated lean beef meat pieces

Samples (12 per group)	**Nesller reaction**							
	day of storage							
	0 d	**1 d**	**2 d**	**3 d**	**4 d**	**5 d**	**6 d**	**7 d**
C (4 ^{0}C) C_1 to C_{12}	**N**	**N**	$C_{9,10}$: **P** $C_{4,5,6,7,8,11}$: **PP** C_{12}: **U** $C_{1,2,3}$: **N**	$C_{9,10}$: **P** $C_{4,5,6,7,8,11}$: **PP** C_{12}: **U** $C_{1,2,3}$: **N**	$C_{8,10,11,12}$: **P** $C_{1,2,3,4,5,6,7,9}$: **N**	C_1 to C_{12}: **P**	-	-
C (25 ^{0}C) C_{13} to C_{24}	**N**	**PP:**4 **U:** 8	C_{13} to C_{24}: **PP**	C_{13} to C_{24}: **P**	-	-	-	-
EG I (4 ^{0}C) EG_1 to EG_{12}	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
EG I (25 ^{0}C) EG_{13} to EG_{24}	**N**	**N**	$E_{23,24}$: **PP** $E_{19,20}$: **U** $E_{13,14,15,16,17,18,21,22}$: **N**	$E_{23,24}$: **PP** $E_{19,20,21}$: **U** $E_{13.14.15.16.17.18,22}$: **N**	$E_{22,23,24}$: **PP** $E_{19,20,21}$: **U** E_{13} to E_{18}: **N**	$E_{22,23,24}$: **P** $E_{19,20,21}$:**PP** E_{13} to E_{18}: **U**	E_{13} to E_{24}: **P**	-

*The qualitative results are presented by four categories of reaction interpretation: Positive (**P**); Poorly Positive (**PP**); Uncertain (**U**), and Negative (**N**).*

As shown in Table 2, only the samples from the EG I stored at 4 ^{0}C remained fresh in the absence of bound ammonia during 7 days of storage. In other experimental groups studied discoloration of meat extract were observed on the first day of storage (4 samples in the control group kept at 25 ^{0}C poorly positive and 8 were expressed uncertain reaction, followed by unspecified unpleasant odor). All 12 samples from the control group, which were stocked

at 4 ^{0}C were strongly altered (P) on the fifth day of storage (followed with discoloration), and when stored at 25 ^{0}C for three days. Changes of the color when performing Nessler reaction in samples EG I kept at 25 ^{0}C began the third day, and on the fifth day only 3 samples (E_{22}, E_{23} and E_{24}) were positive, and sixth day all samples above mention experimental group were positive. Qualitative physic-chemical changes confirmed the strong protective antimicrobial activity of the tested plant extracts.

CONCLUSIONS

The present study confirmed the antimicrobial efficiency of the ethanol extract of the Serbian herb *Kitaibelia vitifolia*, and benefits for the sustainability and safety of beef meat (improving microbiological quality and prolonging the shelf-life of the beef to day seven of examination). Above mentioned extract showed relatively high antimicrobial activity against all the tested bacteria and fungi, and may protect consumers from the hazards of food-borne illness. The present study suggests that the extract of *K. vitifolia* is a potential source for the food industry as a natural antibacterial agent.After this screening, further work should be performed to describe the antimicrobial activities in more detail as well as its activity *in vivo*.

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