Profiles of Antibacterial Activities of Consecutive Extracts from Naked Oat against Several Food-Related Bacteria

Dong-Lin Hao[1], Qing-Ping Hu[*1], Jiang-Lian Duan[2], Jian-Guo Xu[2]

[1] College of Life Sciences, Shanxi Normal University, 1 Gongyuan Street, Linfen, China

[*1] College of Life Sciences, Shanxi Normal University, 1 Gongyuan Street, Linfen, China

Corresponding author's email: E-mail: 1046887727 {at} qq.com

[2] College of Food Sciences, Shanxi Normal University, 1 Gongyuan Street, Linfen, China

ABSTRACT—*The profiles of total phenolic content (TPC) and antibacterial activities of consecutive extracts from naked oat against several food-related bacteria were investigated in this paper. The results showed that the extracting solvent and fractional extraction significantly affected TPC, therefore influenced antibacterial activities of oat extracts. In our study, the ethyl acetate extracts from oats had the highest content of total polyphenols, followed by ethanol, chloroform, and water extracts, the lowest for petroleum ether extracts. No inhibitory effects* of *water extract on the tested bacteria was found, while other extracts from oats showed varying degrees of antibacterial activity against tested bacteria. Among these extracts, the ethyl acetate extracts had the highest antibacterial activities against the tested bacteria, followed by ethanol, chloroform, and petroleum ether extracts. These results indicated that selective extraction from natural sources, by an appropriate solvent or extraction method, is important for obtaining fractions with high antibacterial activity. In addition, though the exact mode of action of the ethyl acetate extracts on bacteria is still not clear, we concluded that one mechanism of action of extracts from oats against B. subtilis and S. dysenteriae was that ethyl acetate extracts disrupt the call wall based on SEM observations.*

Keywords—Naked oat, Extraction solvents, Consecutive extracts, Antibacterial activity

1. INTRODUCTION

In recent years, food poisoning and food spoilage caused by microorganisms are still the most important issues facing the food industry and consumers, and there has been a dramatic increase throughout the world in the number of reported cases of food-borne illness (Sokmen *et al*., 2004; Shan *et al.*, 2007). For many years, a variety of different chemical and synthetic compounds has been made to prevent oxidation and control microbial growth, and to reduce the incidence of food poisoning and spoilage. However, consumers have grown concerned about the side effects of synthetic chemicals and want safer materials for preventing and controlling oxidation and pathogenic microorganisms in foods (Alzoreky & Nakahara 2003). Plants can be an excellent source of natural antibacterial agents and can be effectively used in the food industry to preserve the quality and improve the shelf-life of food products (Tiwari *et al.*, 2009). In addition, as in most of the cases, plants or their extracts are believed to be safe, and non-toxic to humans (Burt 2004; Rymbai *et al.*, 2011).

Oat, a cereal for human or animal consumption, has received increased interest because of its excellent health-related properties, such as high contents of solube dietary fibre and well-balanced protein, energy in the form of carbohydrate and oil, and several vitamins and minerals (Petkov *et al.*, 2001). In addition, oats contained abundant antioxidant compounds such as tocols (Emmons *et al.*, 1999), phytic acid (Miller *et al.*, 1980), sterols (Moreau *et al.*, 1996), phenolic compounds and so on (Peterson 2001). Phenolic compounds exhibited a wide range of differing biological effects, such as antioxidant, anti-inflammatory, antiallergic and anti-carcinogenic activities (Peterson *et al.*, 2002; Chen *et al.*, 2004; Sur *et al.*, 2008). Some studies also reported the antimicrobial activity of extracts from the oats (Shin *et al.*, 2005; Bahraminejad *et al.*, 2008; Sørensen *et al.*, 2010). However, these informations are still limited; little work has been reported on the antibacterial properties of different consecutive extracts from naked oats (*Avena nuda* L.) on the growth of food-related bacteria. Therefore, the objective of this work was to investigate the antibacterial activities of consecutive extracts from oats against several food-borne pathogens, which would provide some foundational information for the developing and application of oats.

2. MATERIALS AND METHODS

2.1 Plant materials and chemicals

A naked oat cultivars (*Avena nuda* L.), Bayou I , were used in the study. The cultivars were all grown in 2012 in bases for growing organic oat, Shanxi, China, and they are the main commercial cultivars in local area. The harvested oat grains were dried to about 12% moisture.

Dimethyl sulfoxide (DMSO), Nutrient agar (NA), nutrient broth (NB) and tryptone soy agar mediums were from Beijing Aoboxing Bio-tech Co. Ltd. (Beijing, China). Other chemicals used were all of analytical grade.

2.2 Microbial strains and culture

The antimicrobial activity of extracts was tested against seven different microorganisms. Three Gram-positive strains were *Staphylococcus aureus* ATCC 25923, *Staphylococcus albus* ATCC 8799, and *Bacillus subtilis* ATCC 6051. Three Gram-negative bacteria were *Salmonella typhimurium* ATCC 19430, *Shigella dysenteriae* CMCC (B) 51252 and *Escherichia coli* ATCC 25922. The strains were provided by the College of Life Science, Shanxi Normal University, and cultured at 37 °C on NA or NB mediums.

2.3 Preparation of extracts

Five solvents having different polarity, including petroleum ether, chloroform, ethyl acetate, ethanol, and water were used to extract consecutively. The dried oats were finely ground with a micro plant grinding machine (FZ102; Tianjin Taisite Instruments, Tianjin, China), and the powder was sifted by 60-meshes. The powder (100 g) was extracted with 1500 mL petroleum ether (30–60 °C) and shaken with a laboratory rotary shaker at 150 rpm for 8 h at 30 °C, and then the homogenates were centrifuged for 10 min at 4 ^{o}C and 5 000 g in a centrifuge (Eppendorf 5417R, Germany). The residue was extracted with ether and ethyl acetate respectively in the same way. The residue was further extracted successively with ethanol (1500 mL × 2 times) and water (1500 mL × 2 times) in shaking water bath at 40 °C for 8 h. All of five solutions were evaporated and dried under vacuum (below 40 °C), to yield the petroleum ether, chloroform, ethyl acetate, ethanol, and water extracts, respectively.

2.4 Determination of total phenolic content (TPC)

Total phenolic content was determined as described by Rebey et al. (2012) with slight modifications. An aliquot (0.1 mL) of diluted extracts, 2.8 mL of deionized water and 0.1 mL of 1.0M Folin-Ciocalteu reagent were mixed and stirred. After 8 min, 2 mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The absorbance of the reaction mixtures was measured at 765 nm wavelength after incubation for 2 h at room temperature. Gallic acid was used for calibration of the standard curve and total phenolic content was expressed as milligram gallic acid equivalent per gram dried extracts (mg GAE/g). All extracts were tested in triplicates.

2.5 Antibacterial activity

Different extracts were redissolved in 20% DMSO and sterilized by filtration through 0.22 μm Millipore filters. Antimicrobial tests were then carried out by the Oxford cup method (Wang *et al.*, 2006) using 100 μL of suspension containing 2×10^6 colony forming units (CFU)/mL of bacteria determined by blood count assay spread on nutrient agar (NA) medium. Oxford cups (6 mm in diameter) were placed on the inoculated agar, and then 100 μL of extracts was added with a micropipette. The diameter of inhibition zone (DIZ) was measured after 24h of incubation at 37 °C, and 20% DMSO was used as a negative control. Tests were performed in triplicate.

2.6 Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC)

MIC and MBC were determined according to the method described by Diao et al. (2013) with minor modifications. Briefly, stock solution of extracts was prepared in 20% DMSO. Two fold serial dilutions of extracts were filtered through 0.22 μm Millipore filters and prepared in sterile NB medium. To each tube, 50 μL of the inoculum containing approximately 2×10^6 CFU/mL microorganisms determined by blood count assay were added. A control test containing inoculated broth supplemented with only 20% DMSO was also performed. The tubes were then incubated at 37 °C and examined for evidence of the growth. The MIC was determined as the lowest concentration of extracts that demonstrated no visible growth for incubating for 24 h, while the MBC was the lowest concentration of the test extracts that showed no visible growth in the culture incubating at 37 °C for 48 h.

2.7 Kill-time analysis

The kill-time curve assay method was used to investigate the bactericidal effects of the extract according to the technique described by Joray et al. (2011). The cultivation with the extract was done the same as the above MIC assay and controls containing only 20% DMSO were simultaneously run. At selected time intervals, samples from the test culture were taken, serially diluted in sterile water, and plated in Plate Count Agar (PCA) medium. All plates were then incubated for 24 h at 37 °C, and CFU were counted.

2.8. Scanning electron microscope (SEM)

To determine the efficacy of the extracts and the morphological changes of strains, SEM observation was performed on the tested bacteria. The bacteria cells were incubated in nutrient broth at 37 °C for 10 h. The suspensions were added 0×, and 1×MIC of extracts, respectively; control culture was left untreated. Next the suspensions were incubated at 37 °C for 4 h respectively, and then the suspensions were centrifuged. The precipitated cells were washed twice with 0.1 M PBS (pH 7.4) and fixed with 2.5% (v/v) glutaraldehyde in 0.1 M PBS overnight at 4°C. After this, the cells were dehydrated using sequential exposure per ethanol concentrations ranging from 30-100% and the ethanol was replaced by tertiary butyl alcohol at last. Then, cells after centrifugation were dried at "critical point" in liquid CO_2 under 95 bar pressure, and samples were gold-covered by cathodic spraying. Finally, morphology of the bacterial cells was observed on a scanning electronic microscope (JSM-7500F, JEOL Ltd., Japan).

2.9 Statistical analysis

All results are expressed as mean ± SD (n=3). One-way analysis of variance (ANOVA) and Duncan's test were performed with significant level being considered at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 TPC profiles of consecutive extracts

The TPC of different consecutive extracts from oats were given in Table 1. Results showed that TPC of different extracts varied considerably and ranged from 20.54 to 262.25 mg GAE/g extracts, respectively, for petroleum ether and ethyl acetate. With respect to TPC, solvents used in the present study could be classified in the following decreasing order: ethyl acetate > ethanol > chloroform > water > petroleum ether. Generally speaking, differences in the content of phytochemicals from different solvent extracts may be come from differences in the polarity of solvents. However, these results were inconsistent with previous studies (Cheok *et al*., 2012; Rebey *et al*., 2012; Turkmen *et al*., 2006). These differences in TPC from oat may be concerned in the cultivated varieties, and extraction methods (Dimberg *et al*., 1996; Emmons & Peterson 2001). On the other hand, the effect of solvents on extraction was also possibly influenced by dielectric constant, chemical structure of organic solvents (Cheok *et al*., 2012) as well as chemical properties of plant phytochemicals (Jayaprakasha *et al*., 2003).

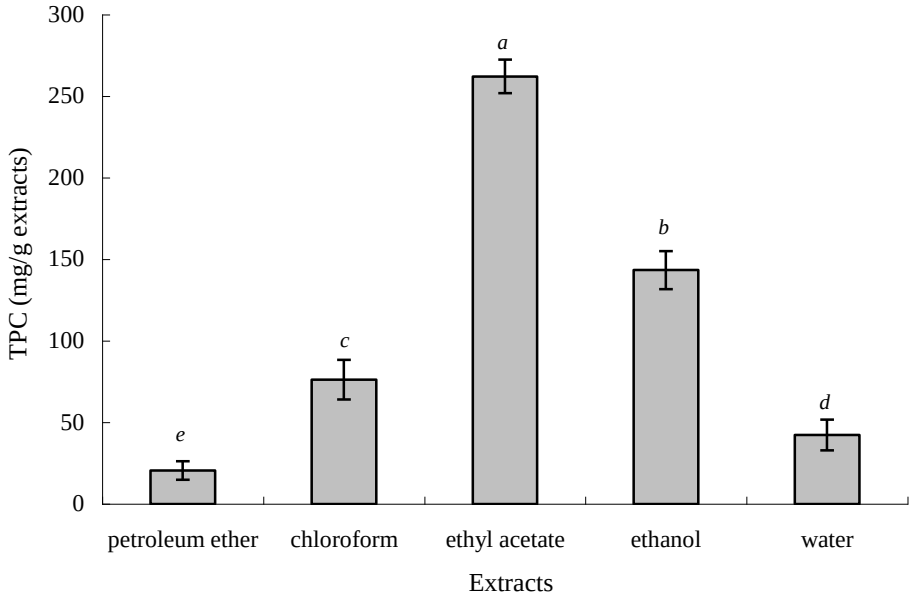

Figure 1: The TPC of different consecutive extracts

3.2 DIZ profiles of consecutive extracts

The DIZ values of the different consecutive extracts against tested strains were presented in Table 2. No inhibitory effects of water extract on each of the tested bacteria was found, while other extracts showed varing degrees of antibacterial activity against tested Gram-positive bacteria and Gram-negative bacteria. The DIZ values for all tested bacterial strains were in the range of 7.8-19.3mm. Compared with other extracts, ethyl acetate extracts exhibited the best antibacterial activity against each of tested bacteria, followed by ethanol, chloroform, and petroleum ether extracts, which may be associated with bioactive constituents because some studies reported that the polyphenols and flavonoids

contribute to the antibacterial activities of plant extracts (Negi *et al*., 2005). The DIZ of *B. subtilis* and *S. dysenteriae* was the maximum value for tested Gram-positive bacteria and Gram-negative bacteria respectively, which may be associated with the genetic characteristics and growth characteristics of different bacteria.

Table 1: Antibacterial activities of different consecutive extracts from oats

	DIZ (mm)					
	S. aureus	*S. albus*	*B. subtilis*	*S. typhimurium*	*S. dysenteriae*	*E. coli*
petroleum ether	7.8±0.4 dB	8.3±0.2 dB	9.4±0.6 dA	9.0±0.3 cA	9.5±0.4 dA	8.1±0.2 cB
chloroform	9.4±0.9 cB	11.0±0.5 cB	12.5±0.8 cA	13.5±0.7 bA	13.1±0.5 cA	12.7±0.8 bA
ethyl acetate	19.3±1.0 aA	19.0±0.5aA	20.8±0.5 aA	17.8±0.7 aB	18.5±1.6 aA	16.7±0.6 aB
ethanol	15.3±0.4 bB	15.2±0.5 bB	17.2±0.6 bA	15.1±0.4 bB	16.9±0.4 bA	14.3±0.3 bC
water	NI [a]	NI	NI	NI	NI	NI

Values are represented as mean ± standard deviation of triplicates; Different small letters within the same column indicate statistically significant differences between the means of different solvent extracts against the same tested strains at P < 0.05; Different upper case letters within the same row indicate statistically significant differences between the means of the same extract against different tested strains at P < 0.05.
[a] *Not inhibition.*

3.3 MIC and MBC profiles of consecutive extracts

The MIC and MBC values for tested bacterial strains were in the range of 31.2-1000 µg/mL (Table 2), respectively. Unfortunately, the MIC and MBC values of some extracts have not been gained when the concentration of extracts reached the maximum in method system tested. Of these bacteria, ethyl acetate extracts had the lowest MIC ranging from 31.2 to 125 µg/mL and MBC ranging from 31.2 to 250 µg/mL against bacteria, followed by ethanol, and chloroform extracts, the lowest for water extracts, which was supported by the results of DIZ assay. These results indicated that ethyl acetate extracts had the best antibacterial effect against both tested bacteria, especially against *B. subtilis* and *S. dysenteria* in Gram-positive bacteria and Gram-negative bacteria respectively. Based on the sensitivity of the test food-related bacteria, one Gram-positive (*B. subtilis*) and one Gram-negative bacteria (*S. dysenteria*) were selected as the model organisms for further study the effect of the ethyl acetate extracts from *oats* on the viable counts of tested bacterial pathogen in the present study.

Table 2: The MIC and MBC values (µg/mL) of different consecutive extracts from oats

	Petroleum ether		Chloroform		Ethyl acetate		Ethanol		Water	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	1000	ND [a]	500	1000	62.5	125	62.5	125	ND	ND
S. albus	1000	ND	500	500	62.5	62.5	62.5	125	ND	ND
B. subtilis	500	1000	250	250	31.2	31.2	31.2	62.5	ND	ND
S. typhimurium	500	1000	250	500	125	250	250	250	ND	ND
S. dysenteriae	500	1000	250	500	31.2	62.5	62.5	125	ND	ND
E. coli	1000	ND	250	500	125	250	250	500	ND	ND

[a] *Not detect.*

3.4 Kill-time analysis

The effect of the ethyl acetate extracts on the viable counts of tested bacterial pathogen is shown in Figure 2. As observed in Figure 2, compared to the control, susceptible *B. subtilis* treated with the ethyl acetate extracts at the 1×MIC value showed a decrease, and the number of viable cells decreased by 46% from 6.15 to 3.32 $\log_{10}$ CFU/mL at the cultivation time of 24 h. Unlike the changing trend of the number of viable cells at 1×MIC, in treatments at 2×MIC, the number of viable cells decreased obviously from the first hour after cultivation and decreased by 97.55% to 0.15 $\log_{10}$ CFU/mL over 24 h of incubation. Similarly, the effect of the ethyl acetate extracts on the viable counts of *S. dysenteria* was similar to the result on *B. subtilis.* Somewhat differently, the extent of change was not in keeping with the former. These results showed that the treatment time and concentration of extracts had great influences on antibacterial effects.

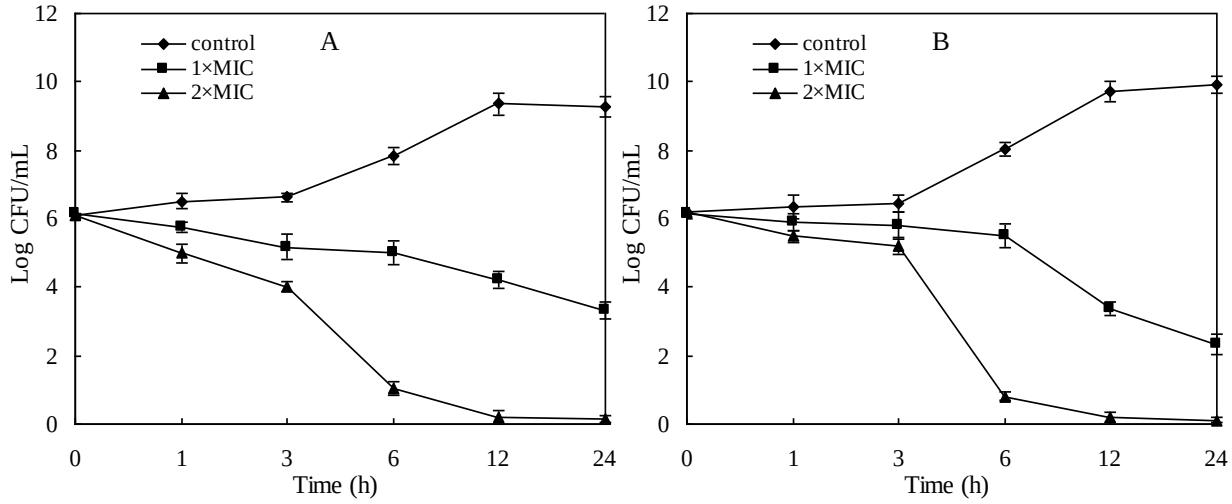

Figure 2: Effect of the extracts on the viability of the tested *B. subtilis* (A) *and S. dysenteria* (B)

3.5 Electron microscope observation

The *B. subtilis and S. dysenteriae* were treated with the ethyl acetate extracts from oats at 1×MIC for 4 h respectively, and then the morphological and physical changes of treated strains were observed by SEM. Figure 3 showed the SEM images of the treated and untreated bacteria. These images directly illustrated the destructive effects of the ethyl acetate extracts on the tested bacteria. The surfaces of the treated strains underwent some morphological changes compared with the untreated controls. Untreated *B. subtilis* cells were rod shaped, regular, intact and showed a smooth surface (Figure 3, A0), while bacterial cells treated with extracts became deformed, pitted, and shriveled (Figure 3, A1). Similar changes in *S. dysenteriae* were found, and bacterial cells also became deformed, pitted, shriveled after treating with the ethyl acetate extracts (Figure 3, B0 and B1), which indicated that the ethyl acetate extracts from oats may have severe effects on the cell wall and cytoplasmic membrane.

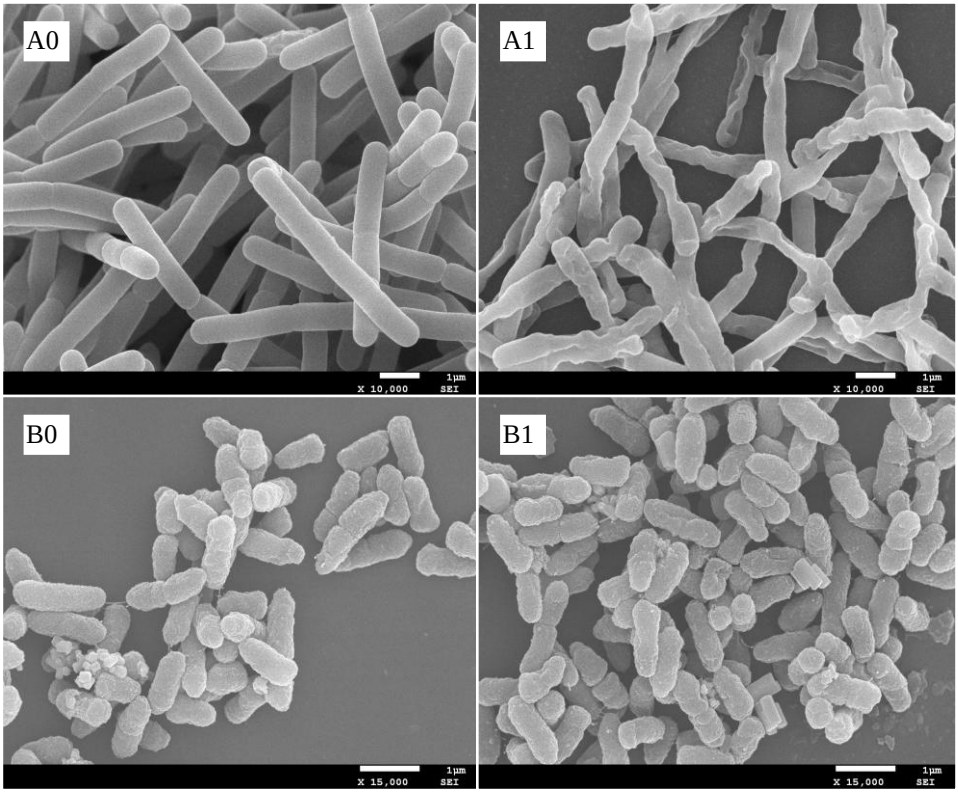

Figure 3: The SEM photography of tested strains for A0 and B0, untreated *B. subtilis and S. dysenteriae*, respectively; A1 and B1, *B. subtilis and S. dysenteriae* treated with the ethyl acetate extracts, respectively

4. CONCLUSION

The extracting solvent and fractional extraction significantly affected total polyphenols and antibacterial activities of oats. In our study, the ethyl acetate extracts from oats had the highest content of total polyphenols, followed by ethanol, chloroform, and water extracts, the lowest for petroleum ether extracts. No inhibitory effects of water extract on the tested bacteria was found, while other extracts from oats showed varing degrees of antibacterial activity against tested bacteria. Among these extracts, the ethyl acetate extracts had relatively higher antibacterial activities against the tested bacteria, followed by ethanol, chloroform, and petroleum ether extracts. These results indicated that selective extraction from natural sources, by an appropriate solvent or extraction method, is important for obtaining fractions with high antibacterial activity. In addition, though the exact mode of action of the ethyl acetate extracts on bacteria is still not clear, we concluded that one mechanism of action of extracts from oats against *B. subtilis and S. dysenteriae* was that ethyl acetate extracts disrupt the call wall based on SEM observations. Therefore, further research on the chemical compositions, mechanisms of action as well as the effects of extracts from oats on other food spoilage and poisoning bacteria is still necessary.

5. ACKNOWLEDGEMENT

This work was financially supported by a project of the Natural Science Foundation of Shanxi Province, China (project no. 2012011031-3), and a Program for the Top Young Academic Leaders of Higher Learning Institutions of Shanxi.

6. References

1. Alzoreky NS, Nakahara K. 2003. Antimicrobial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology* **80**: 223–230.
2. Bahraminejad S, Asenstorfer RE, Riley IT, Schultz CJ. 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.). *Journal of Phytopathology* **156**: 1–7.
3. Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology* **94**: 223–253.
4. Chen CY, Milbury PE, Kwak HK, Collins FW. 2004. Avenanthramides and phenolic acids from oats are bioavailable and act synergistically with vitamin C to enhance hamster and human LDL resistance to oxidation. *Journal of Nutrition.* **134**: 1459–1466.
5. Cheok CY, Chin NL, Yusof YA, Law CL. 2012. Extraction of total phenolic content from *Garcinia mangostana* Linn. hull. I. Effects of solvents and UV–Vis spectrophotometer absorbance method. *Food and Bioprocess Technology* **5**: 2928-2933.
6. Diao WR, Hu QP, Feng SS, Li WQ, Xu JG. 2013. Chemical composition and antibacterial activity of the essential oil from green huajiao (*Zanthoxylum schinifolium*) against selected foodborne pathogens. *Journal of Agricultural and Food Chemistry* **61**: 6044−6049.
7. Dimberg LH, Molteberg EL, Solheim R, Frølich W. 1996. Variation in oat groats due to variety, storage and heat treatment. I: Phenolic compounds. *Journal of Cereal Science* **24**: 263–272.
8. Emmons CL, Peterson DM. 2001. Antioxidant activity and phenolic content of oat as affected by cultivar and location. *Crop Science* **41**: 1678–1681.
9. Emmons CL, Peterson DM, Paul GL. 1999. Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. in vitro antioxidant activity and contents of phenolic and tocol antioxidants. *Journal of Agricultural and Food Chemistry* **47**: 4894–4898.
10. Jayaprakasha GK, Selvi T, Sakariah KK. 2003. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International* **36**: 117–122.
11. Joray MB, del Rollán MR, Ruiz GM, Palacios SM, Carpinella MC. 2011. Antibacterial activity of extracts from plants of central Argentina--Isolation of an active principle from *Achyrocline satureioides*. *Planta Medica,* **77**: 95–100.
12. Miller GA, Youngs VL, Oplinger ES. 1980. Environmental and cultivar effects on oat phytic acid concentration. *Cereal Chemistry* **57**: 189–191.
13. Moreau RA, Powell MJ, Hicks KB. 1996. Extraction and quantitative analysis of oil from commercial corn fiber. *Journal of Agricultural and Food Chemistry* **44**: 2149–2154.
14. Negi PS, Chauhan AS, Sadia GA, Rohinishree YS, Ramteke RS. 2005. Antioxidant and antibacterial activities of various seabuckthorn (*Hippophae rhamnoides* L.) seed extracts. *Food Chemistry* **92**: 119–124.
15. Petkov K, Biel W, Kowieska A, Jaskowska I. 2001. The composition and nutritive value of naked oat grain (*Avena sativa var. nuda*). *Journal of Animal and Feed Sciences* **10**: 303–307.
16. Peterson DM. 2001. Oat antioxidants. *Journal of Cereal Science* **33**: 115–129.

17. Peterson DM, Hahn MJ, Emmons CL. 2002. Oat avenanthramides exhibit antioxidant activities in vitro. *Food Chemistry* **79**: 473–478.
18. Rebey IB, Bourgou S, Debez IBS, Karoui IJ, Sellami IH, Msaada K, Limam F, Marzouk B. 2012. Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. *Food and Bioprocess Technology* **5**: 2827–2836.
19. Rymbai H, Sharma RR, Srivasta M. 2011. Bio-colorants and its implications in health and food industry–a review. *International Journal of Pharmacological Research* **3**: 2228–2244.
20. Shan B, Cai Y-Z, Brooks JD, Corke H. 2007. Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): activity against foodborne pathogenic bacteria. *Journal of Agricultural and Food Chemistry* **55**: 5484–5490.
21. Shin MS, Lee S, Lee KY, Lee HG. 2005. Structural and biological characterization of aminated-derivatized oat *β*-glucan. *Journal of Agricultural and Food Chemistry* **53**: 5554-5558.
22. Sokmen A, Gulluce M, Akpulat HA, Daferera D, Tepe B, Polissiou M, Sokmen M, Sahin F. 2004. The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. *Food Control* **15**: 627–634.
23. Sur R, Nigam A, Grote D, Liebel Frank, Southall MD. 2008. Avenanthramides, polyphenols from oats, exhibit anti-inflammatory and anti-itch activity. *Archives of Dermatological Research* **300**: 569–574.
24. Sørensen HP, Madsen LS, Petersen J, Andersen JT, Hansen AM, Beck HC. 2010. Oat (*Avena sativa*) seed extract as an antifungal food preservative through the catalytic activity of a highly abundant class I chitinase. *Applied Biochemistry and Biotechnology* **160**: 1573–1584.
25. Tiwari BK, Valdramidis VP, Donnel CPO, Muthukumarappan K, Bourke P, Cullen PJ. 2009. Application of natural antimicrobials for food preservation. *Journal of Agricultural and Food Chemistry* **57**: 5987–6000.
26. Turkmen N, Sari F, Sedat Velioglu Y. 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. *Food Chemistry* **99**: 835–841.
27. Wang GL, Tang JH, Jiang D, Fang HJ, Liu ZB. 2006. Bacteriostatic action and mechanism of *Sophora flavescens* ait on *Escherichia coli* 01 C84010. *Scientia Agricultura Sinica* **39**: 1018–1024.