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Antimicrobial Activity of Various Extracts from Different Parts of *Amaranthus mangostanus*

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Amaranthus mangostanus is a green vegetable which is also used as sudorific, febrifuge, emollient, lactogogue and a specific treatment for colic. This study was aimed to determine antibacterial and antifungal activities of the methanol, dichloromethane, petroleum ether and ethyl acetate extracts from different parts of *A. mangostanus* against microbes that cause plant or human diseases and food contamination. At concentrations ranging from 40-100 mg/mL, ethyl acetate extracts from leaves and stems exhibited concentration-dependent inhibition against *Pseudomonas solanacearum* Smith, *Acidovorax avenae* subsp. *A. Citrulli*, *Rhizoctonia solani*, *Colletotrichum capsici* (syd.) Butl, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*, but not *Magnaporthe grisea*, *Alternaria alternata* Keissler, *Phytophtora parasitica* var. nicotianae Tucker and *Fusarium graminearum* Sehw., with MIC values of 519-713 and 463-697 mg/mL, respectively, whereas the ethyl acetate extract from roots only showed antibacterial activity against *P. solanacearum* Smith with an MIC of 815 mg/mL. Ethyl acetate extracts from leaves and stems also showed inhibitory effect on the fungus *R. solani*, with MICs of 686 and 734 mg/mL, respectively. Consequently the information on the antimicrobial functions of the extracts can shed light on the discovery of natural products for management of plant and human infectious diseases.

Key Words: *Amaranthus mangostanus*, extracts, Antimicrobial activity.

INTRODUCTION

Plants have provided a source of inspiration of new therapeutic agents for human as well as agricultural agents for agricultural production, as a considerable proportion of the world's population depends on the traditional medicine systems for various human or crop diseases. Their role is twofold; namely they provide key chemical structure for the development of new antimicrobial drugs and also as phytomedicines to be used for the treatment of diseases in human and crops[1,2] . Although advances have been made in pharmacology and synthetic organic chemistry, this reliance on natural products, particularly on plants, remains largely unchanged. Furthermore, it is expected that the wide use and extension in the utilization of such local agricultural products would increase and stabilize the income of farmers in the rural areas. It is well established that some plants contain compounds able to inhibit microbial growth[3]. These plant-derived compounds have different structures and actions when compared with antimicrobials conventionally used to control the microbial growth and survival. The potential antimicrobial properties of plants are related to their ability to synthesize by secondary metabolism several chemical compounds of relatively complex structures with antimicrobial activity, including tannins, phloba-tannins, alkaloids, coumarins, cardiac glycosides, terpenes, phenylpropanes, organic acids, flavonoids, isoflavonoids and saponins[3,4]. Because of the emerging development of drug resistance by pathogenic microorganism against synthetic antibiotics; attention has now shifted to extracts of biologically active components isolated from plant species used as herbal medicine. Medicinal plants may represent a new source of antibacterial, antifungal and antiviral agents.

Amaranth is a general term for the plants that belong to the genus *Amaranthus*, which are ancient American food crops recently rediscovered. It is an annual dicotyledonous plant and commonly considered as a pseudo-cereal. Currently *Amaranthus* spp. attracts intensive interest due to theirs higher nutritional value than many other crops. Besides the nutritional and rheological properties of the substances from their seeds, various parts of amaranth plants may function physiologically in human due to protease inhibitors, antimicrobial peptides, lectins, antioxidant and anticancer compounds present in the diet prepared from them[5-8]. Recently, the leaf and seed of amaranth are found to be rich in several phytonutrients that may play an important role in inhibiting both free radicals and oxidative chain reactions within tissues

and membranes[9-11]. Control of microorganism is crucial in control of crop or human diseases and food safety. Many studies have shown that *Amaranthus* has antimicrobial activity. The extract of whole plant of *A. hybridus* shows antibacterial activity against both gram-negative and gram-positive bacteria[12]. In terms of chemistry, several antifungal peptides and lectins were identified in leaves or seeds of *A. caudatus*, *A. viridis* and *A. retroflexus* and showed inhibitory activity on various pathogenic fungi[13-16]. As mankind have made use of the antimicrobial properties of these plants in the traditional medicine for centuries without any signs of toxicity, they play important role in primary agricultural systems in the developing world and are becoming increasingly popular in the developed countries.

As an important member of the genus, *A. mangostanus* has been reported to have several pharmacological properties. Extracts of the leaf had also been used in the treatment of menstrual disorders. The plant is used as a sudorific and febrifuge and is recommended for eruptive fevers. The leaves are considered a good emollient, lactogogue and a specific treatment for colic. Externally, the bruised leaves are applied locally to treat eczema. However, no work has been done on the antimicrobial activity of *A. mangostanus*, especially the inhibitory effect on the microbes that cause plant diseases. Therefore, this study was for the first time aimed to determine the antimicrobial activity of the methanol, dichloromethane, petroleum ether and ethyl acetate extracts of the species. Information on the antimicrobial functions of the extracts from the species may contribute to the development of new agents for management of plant and human infectious diseases and improvement of food habits and public health.

EXPERIMENTAL

Fresh leaves of *A. mangostanus* were collected from Hunan Agricultural University in Changsha. Botanic identification was performed at College of Biosafety Science and Technology, Hunan Agricultural University. Voucher samples were prepared and deposited in the herbarium of the College of Biosafety Science and Technology, Hunan Agricultural University. The fresh leaves were fresh to milled in the laboratory at room temperature of 25-26 ºC and the powdered sample were then stored in an air-tight container at 4 ºC for further use.

Test microorganisms: The microorganisms used for antimicrobial sensitivity testing included *Pseudomonas solanacearum* Smith, *Acidovorax avenae* subsp. Citrulli, *Rhizoctonia solani*, *Magnaporthe grisea*, *Alternaria alternata* Keissler, *Phytophtora parasitica* var. nicotianae Tucker, *Fusarium graminearum* Sehw, *Colletotrichum capsici* (syd.) Butl, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*. The microorganisms were sourced from Agricultural Culture Collection of China.

Preparation of solvent extracts: The pulverized specimens of fresh *A. mangostanus* leaves,stems and roots were dried at 40 ºC and then sequentially extracted with petroleum ether, ethyl acetate, dichloromethane and methanol for 48 h, respectively. The preparation of each solvent was then filtered through active charcoal and the solvent was evaporated at 42 ºC *in vacuo*. The resultant residues were then dried in a drying cabinet.

Antimicrobial activity screening and MIC test: The antimicrobial activity of the extracts was tested using agar diffusion method. The active extract extracts were serially diluted in the respective solvents used for their extraction to concentrations of 10, 20, 40, 80 and 100 of the undiluted concentration.

Fungi susceptibility testing was conducted on PDA solid medium. Briefly, suspensions of tested pathogenic fungi were prepared and dispensed uniformly in the culture plates. The plates were incubated at 28 ºC for 24 h to allow the mycelia to grow. Small wells (φ = 5 mm) were cut in the medium along the two sides of the mycelia. Diluted solutions of different extracts (50 µL) were loaded in the wells at one side and the corresponding diluting solvents (50 µL) were loaded in the wells at the other side. The diameters of the inhibition zones were measured by cross-section method after 24-48 h of incubation at 28 ºC. Bacterial susceptibility testing was conducted in NA plates. Freshly cultured bacteria suspensions in NA broth were standardized to a cell density of 1.0×10^8 cfu/mL. Prepared suspensions (60 µL) of bacteria were spread over the plates before two rows of small wells (ϕ = 5 mm)were cut in the medium. Diluted solutions of different extracts (50 µL) were loaded in the wells in one row and the corresponding diluting solvents (50 µL) were loaded in the wells in the other row. The experiments were conducted in triplicate.

Minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited growth of the microorganism detected visually. Under sterile conditions, solutions of extracts from leaves, roots and stems of *A. mangostanus* at different concentrations (2 mL) were transferred in the sterilized plates and 10 mL dissolved PDA or NA media were added. The solution and the medium in each plate were mixed and solidified when cooling. A fungus inoculum was loaded or a standardized bacterium suspension was spread in the center of each plate. Streptomycin and nystatin were used as positive controls of antibiotic and antifungal drugs, respectively. All tests were carried out in triplicate. The plates were incubated for 48 h and the growth of the microbial colonies was observed. When no growth of the microbial tested was observed in the plate, the lowest concentration of an extract applied was determined as MIC.

Statistical analysis of the results: The results were expressed as means ± standard error (SE). Analysis of variance was conducted in SPSS by using Duncan's multiple range test for single-factor completely randomized experiments.

RESULTS AND DISCUSSION

Antifungal activity of extracts with different polarity from different parts: There was a large variability in the antimicrobial activities displayed in the screening of this study (Table-1). The results show that the microorganisms inhibited by the extracts were *Pseudomonas solanacearum* Smith, *Acidovorax avenae* subsp. *A. Citrulli*, *Rhizoctonia solani*, *Colletotrichum capsici* (syd.) Butl, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*. But the extracts did not inhibit *Magnaporthe grisea*, *Alternaria alternata* Keissler,

TABLE-1
ANTIMICROBIAL ACTIVITY OF EXTRACTS WITH DIFFERENT POLARITY OBTAINED FROM DIFFERENT PARTS OF *A. mangostanus*

Part	Solvent	Bacteria		Fungi						Bacteria causing food contamination		
		P.solanacearum Smith	*A.Citrulli*	*R.solani*	*M.grisea*	*A.alternata*	*P.nicotianae Tucker*	*F. graminearum Sehw*	*C.capsici Butl*	*P.aeruginosa*	*B.cereus*	*E.coli*
Leaf	Methanol	–	–	–	–	–	–	–	–	–	–	–
	Dichloromethane	–	–	–	–	–	–	–	–	–	–	–
	Petroleum ether	–	–	–	–	–	–	–	–	–	–	–
	Ethyl acetate	+	+	+	–	–	–	–	–	+	+	+
Stem	Methanol	–	–	–	–	–	–	–	–	–	–	–
	Dichloromethane	–	–	–	–	–	–	–	–	–	–	–
	Petroleum ether	–	–	–	–	–	–	–	–	–	–	–
	Ethyl acetate	+	+	+	–	–	–	–	+	+	+	+
Root	Methanol	–	–	–	–	–	–	–	–	–	–	–
	Dichloromethane	–	–	–	–	–	–	–	–	–	–	–
	Petroleum ether	–	–	–	–	–	–	–	–	–	–	–
	Ethyl acetate	+	–	–	–	–	–	–	–	–	–	–

Note: + Represents presence of antimicrobial activity; – represents absence of antimicrobial activity.

Phytophtora parasitica var. nicotianae Tucker and *Fusarium graminearum* Sehw. The ethyl acetate extracts of *A. mangostanus* leaves and stems contained various types of pharmacologically active compounds with antimicrobial activity. The extracts of *A. mangostanus* leaves and stems have a strong antimicrobial activity on pathogenic bacteria tested. By contrast, they did not show inhibitory effect on pathogenic fungi. The current study also proved that the root ethyl acetate extract of *A. mangostanus* roots had antimicrobial activity against *P. solanacearum* Smith. However, it did not exhibit antimicrobial activity on fungi and other bacteria strains. Other extracts did not show antimicrobial activity against tested pathogens. Based on the above results, the ethyl acetate extracts of *A. mangostanus* leaves and stems may possess potential antimicrobial activity and were therefore subjected to further testing to determine their MICs.

Inhibition effect and minimum inhibitory concentration of ethyl acetate extracts from different parts: The inhibition of ethyl acetate extracts obtained from different parts of *A. mangostanus* on pathogenic bacteria strains is illustrated in Figs. 1-4. The ethyl acetate extracts of different parts of *A. mangostanus* did not show inhibition against all bacteria at the concentrations ranging from 10 to 20 mg/mL. At concentrations from 40-100 mg/mL, the ethyl acetate extracts from different parts of *A. mangostanus* exhibited a larger inhibition zone with the increase of concentration, indicating a concentration-dependent manner of the action. Within a concentration range of 40 to 100 mg/mL, ethyl acetate extracts from leaves and stems exhibited inhibition against all five bacteria that causes plant or human diseases or food contamination (Figs. 1 and 2), while the extract from roots did not show activity. The variance analysis showed that there are significant differences between the effects of ethyl acetate extracts from different parts of *A. mangostanus*. The ethyl acetate leaves extracts of *A. mangostanus* were active against A. Citrulli, *P. solanacearum* Smith, *P. aeruginosa*, *B. cereus* and *E. coli* with an MIC ranging between 524 and 712 mg/mL (Table-2). However, the ethyl acetate extract of *A. mangostanus* roots was

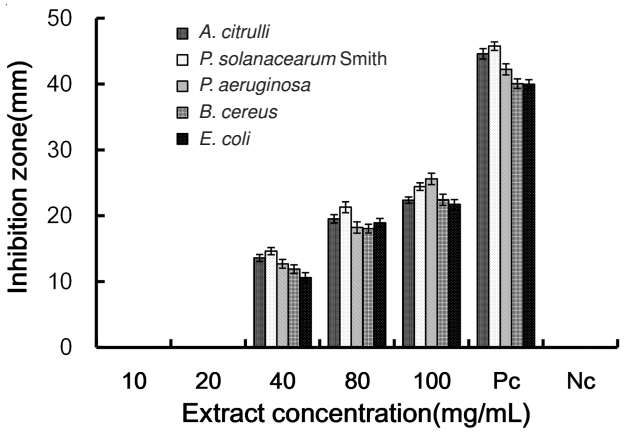

Fig. 1. Inhibition effect of ethyl acttate leaf extracts on several pathogens. Pc: Positive control, Nc: Negative control

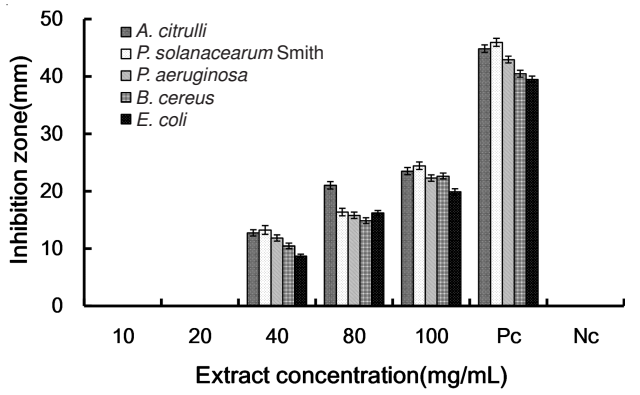

Fig. 2. Inhibition effect of stem ethyl acetate eatracts on several pathogens. Pc: Positive control, Nc: Negative control

only active in inhibition of *P. solanacearum* Smith with an MIC of 815 mg/mL (Fig. 3, Table-2). In addition, ethyl acetate extracts from leaves and stems showed inhibitory effect on the *R. solani*, which causes collar rot and root rot of plants (Fig. 4, Table-3), whereas extracts from other parts of the plant did not displayed inhibition against fungi. As a photosynthetic organ, substances in leaves of *A. mangostanus* may have lower

TABLE-2
MINIMUM INHIBITORY CONCENTRATION (MIC) OF ETHYL ACETATE EXTRACTS OF DIFFERENT PARTS OF *A. mangostanus* ON SEVERAL BACTERIAL PATHOGENS

Position	Pathogens	MIC (mg/mL)	
		Extracts	Positive control
Leaf	*A. citrulli*	698.00 ± 2.00Bb	25.76 ± 1.17ABb
	P. solanacearum smith	527.00 ± 2.65Dd	23.52 ± 0.17Bb
	P. aeruginosa	519.00 ± 4.29Ee	25.93 ± 1.42Abb
	B. cereus	713.33 ± 2.31Aa	23.57 ± 1.63Bb
	E. coli	655.67 ± 2.08Cc	29.02 ± Aa
Stem	*A. citrulli*	583.00 ± 4.68Bc	25.76 ± 1.17ABb
	P. solanacearum smith	463.33 ± 4.51Cd	23.52 ± 0.17Bb
	P. aeruginosa	576.67 ± 4.61Bc	25.93 ± 1.42Abb
	B. cereus	697.00 ± 2.65Aa	23.57 ± 1.63Bb
	E. coli	676.33 ± 1.15Ab	29.02 ± Aa
Root	*A. citrulli*	0	25.76 ± 1.17ABb
	P. solanacearum smith	815.33 ± 0.58Aa	23.52 ± 0.17Bb
	P. aeruginosa	0	25.93 ± 1.42Abb
	B. cereus	0	23.57 ± 1.63Bb
	E. coli	0	29.02 ± Aa

Note: 1: MIC value = mean ± standard error; 2: Different small letters show significant differences ($p < 0.05$). Different capital letters show extremely significant differences ($p < 0.01$).

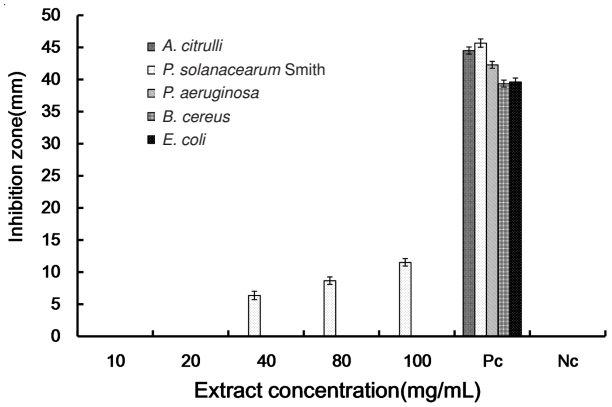

Fig. 3. Inhibition effect of stem ethyl acetate extracts on several pathogens. Pc: Positive control, Nc: Negative control

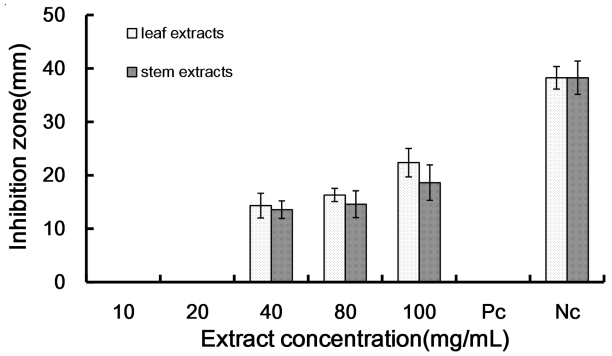

Fig. 4. Inhibition effect of leaf and stem ethyl acetate extracts on fungus pathogens (*Rhizoctonia solani*). Pc: Positive control, Nc: Negative control

MIC than those in stems theoretically. However, present study showed that extracts from stems of *A. mangostanus* had lower MIC than those from leaves. This may be ascribed to different quantity of the photosynthetic products in different developmental periods.

Many plants are not only regarded as traditional pesticide but also agricultural commodities meeting the demand of distant markets. To cope with the challenges arising from the growing market, it is necessary to expeditiously utilize and scientifically validate more plants in agriculture. The emergence of antipesticide resistance in microorganism to synthesized pesticides has necessitated the discovery of new effective and economical agents for the control of disease caused by microbes in agricultural production. It have been suggested that natural products are a preferable option to synthetic ones in terms of pest control agent. Literature indicates the antibacterial activity of plant extract is due to different chemical agents with antimicrobial compounds in the extract[17]. In plants, these secondary metabolites function to attract beneficial and repel harmful organisms, serving as plant protectants and playing an important role in plants' response to environmental changes. In humans, these compounds have beneficial effects including antioxidative, antiinflammatory, immune-stimulatory, antibacterial and antiviral as well as modulatory effects on detoxification enzymes and steroid metabolism[18]. The antimicrobial compounds isolated from the genus *Amaranthus* are mainly peptides or protein derivatives. For instance, several antifungal peptides were identified in leaves and seeds of *A. caudatus* and showed inhibitory activity on pathogenic fungi[13]. A novel lectin from *A. viridis* was found to have antifungal activity[15] and a peptide Ar-AMP from *A. retroflexus* is able to inhibit the growth of various fungi[14]. Rizzello and coworkers[16] found the crude water-soluble extract of amaranth seeds had minimal inhibitory concentration (MIC) of 5 mg of peptides/mL and showed inhibition towards a large number of fungal species isolated from bakeries and isolated four antifungal peptides from the seeds. This indicates that the crude water-soluble

TABLE-3
INHIBITION EFFECT OF ETHYL ACETATE EXTRACTS ON THE FUNGUS *Rhizoctonia solani*

Position	Pathogens	MIC (mg/mL)	
		Extracts	Negative control
Leaf	*R. solani*	686 ± 2.63	21.31 ± 1.22
Stem	*R. solani*	734 ± 1.58	21.31 ± 1.22
Root	*R. solani*	0	21.31 ± 1.22

Note: MIC value = mean ± standard error.

extract of amaranth seeds may be useful in extending the shelf-life of gluten-free and wheat flour breads. These studies suggest that the ethyl acetate extracts of *A. mangostanus* may also contain similar constituents. However, further investigation is needed to identify the active compounds responsible for the antimicrobial activity of different parts of the plant. In addition, the inhibitory activity of the extracts from the plant on other pathogenic microbes such as viruses, nematode and parasitic seed plants remain unstudied. In some literature, extraction has been done to the dried plant material while we had used fresh material in this study. Future work should therefore be conducted to determine the optimal extraction conditions of the active extracts from the plant. In this study, the anti-microbial activity of different parts of the mature plant of *A. mangostanus* was investigated. However, the antimicrobial activity of *A. mangostanus* at different developmental stages remains unknown. The effects of farming methods and fertilizing measure on its antimicrobial activity have also not been investigated. Future research can be focused on these aspects for better understanding of antimicrobial activity of the plant.

Conclusion

The antimicrobial properties of *A. mangostanus* was studied in this work. Ethyl acetate extracts from different parts of *A. mangostanus* had inhibitory effect on a panel of bacteria and a fungus that cause plant or human diseases and food contamination. Information on the antimicrobial functions of the extracts from the species can shed light on the discovery of natural products for plant disease management and improvement of food habits and public health.

REFERENCES

1. P. Aureli, A. Costantini and S. Zolea, *J. Food Protect*., **55**, 344 (1992).
2. M. Abukakar, A. Ukwuani and R. Shehu, *Asian J. Biochem*., **3**, 134 (2008).
3. E.L. Souza, E.O. Lima, K.R.L. Freire and C.P. Sousa, *Braz. Arch. Biol. Technol*., **48**, 245 (2005).
4. J. Matasyoh, Z. Maiyo, R. Ngure and R. Chepkorir, *Food Chem*., **113**, 526 (2009).
5. B. Jayaprakasam, Y. Zhang and M.G. Nair, *J. Agric. Food Chem*., **52**, 6939 (2004).
6. H.A. Sani, A. Rahmat, M. Ismail, R. Rosli and S. Endrini, *Asia Pacific J. Clin. Nutr*., **13**, 396 (2004).
7. K. Chitindingu, A. Ndhlala, C. Chapano, M. Benhura and M. Muchuweti, *J. Food Biochem*., **31**, 206 (2007).
8. C. Silva-Sanchez, A.P. de la Rosa, M.F. Leon-Galvan, B.O. de Lumen, A. de Leon-Rodriguez and E.G. de Mejia, *J. Agric. Food Chem*., **56**, 1233 (2008).
9. L.R. Acevedo and M.S. García, *J. Mexican Chem. Soc*., **51**, 136 (2007).
10. R. Yawadio Nsimba, H. Kikuzaki and Y. Konishi, *Food Chem*., **106**, 760 (2008).
11. Z. Maiyo, R. Ngure, J. Matasyoh and R. Chepkorir, *Afr. J. Biotechnol*., **9**, 3178 (2010).
12. W.G. Cyrus, G.W. Daniel, M.O. Nanyingi, F.K. Njonge and J.M. Mbaria, *Memorias do Instituto Oswaldo Cruz*, **103**, 650 (2008).
13. N. Lyapkova, N. Loskutova, A. Maisuryan, V. Mazin, N. Korableva, T. Platonova, E. Ladyzhenskaya and A. Evsyunina, *Appl. Biochem. Microbiol*., **37**, 301 (2001).
14. A. Lipkin, V. Anisimova, A. Nikonorova, A. Babakov, E. Krause, M. Bienert, E. Grishin and T. Egorov, *Phytochemistry*, **66**, 2426 (2005).
15. N. Kaur, V. Dhuna, S.S. Kamboj, J.N. Agrewala and J. Singh, *Protein Peptide Lett*., **13**, 897 (2006).
16. C. Giuseppe Rizzello, R. Coda, M. De Angelis, R. Di Cagno, P. Carnevali and M. Gobbetti, *Int. J. Food Microbiol*., **131**, 189 (2009).
17. A. Rojas, L. Hernandez, R. Pereda-Miranda and R. Mata, *J. Ethnopharmacol*., **35**, 275 (1992).
18. J.W. Lampe, *Am. J. Clin. Nutr*., **78**, 579S (2003).