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Effects of Aqueous Extracts and Essential Oil from *Cinnamomum cassia* on Rat Colonic Mucosal Morphology and *Bacteroidales*

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Abstract Recently, the preventive effects of *Cinnamomum cassia* on several metabolic diseases, e.g. obesity, diabetes, hypertension, have been reported. However, no literature reported on the interaction of phytochemicals from *C. cassia* and colonic microecology.This study aimed to explore the impact of essential oil and aqueous extracts of *C. cassia* on rat colonic mucosal morphology and microbiota. After 4-week feed, the rats administrated with aqueous extracts of *C. cassia* (AEC) resulted in less body-weight gain (45.33g, $P<0.01$) and those administrated with essential oil of *C. cassia* (EOC) had no difference in weight gain compared with controls (85.33g vs. 87.67g, $P>0.05$). AEC rather than EOC decreased villous area and crypt count of rat colonic mucosa significantly ($P<0.01$). According to the TRFLP analysis, both of the AEC and EOC increased total peak areas of *Bacteroidales* ($P<0.05$), as well as changed terminal restriction fragments in different degrees (mainly 76 bp and 614 bp, $P<0.01$). AEC altered the tested rats' colonic mucosa and community structure of probably obesity-associated gut microbiota (*Bacteroidales*). This change potentially resulted in a decreased energy harvest from diet as the rats gained less body weight. Despite EOC up-regulated the total amount and diversity of colon *Bacteroidales*, its influence on weight gain had scarcely been detected.

Keywords: *cinnamomum cassia, essential oil, aqueous extracts, colon, mucosal morphology, bacteroidales*

1. Introduction

Cinnamomum cassia, also known as Chinese Cinnamon, is commonly used as spice in Chinese cooking due to its specific sweetness and health function [1]. It has also been shown that *C. cassia* possesses a wide variety of biological functions, including anti-oxidant [2], anti-inflammation [3], anti-tumor activity [4], and especially antimicrobial [5] and anti-diabetic effects [6]. Thus it has been used in Chinese medicine for treating dyspepsia, gastritis, blood circulation disturbances, and inflammatory diseases [7]. Aqueous extracts of *C. cassia* (AEC) contains various aromatics, diterpenes, polyphenols (e.g. anthocyan, flavonoid and tannin) [7,8], mucus and carbohydrates [9]; while essential oil of cinnamon (EOC) mainly contains cinnamaldehyde (60%-80%) [10,11], cinnamic acid, cinnamyl alcohol, and coumarin [12].

Studies have suggested that 80%-90% of dietary polyphenols in AEC are not absorbed in small intestine. Instead, they directly reach the large intestine and are metabolized by gut microbiota [13]. The main bioactive components in EOC will be rapidly absorbed from the gut [14]. Numbers of evidences have demonstrated the interaction of phytochemicals and gut microbiota. The gut microbiota has been shown to be essential for the transformation of some phytochemicals e.g. polyphenols [13]. On the other hand, phytochemicals and their derived products can also affect the composition of the gut microbiota [15]. Furthermore, the intestinal bacteria were associated with the colonic mucosal architecture according to the finding that the fructans-containing diet increased colonic crypt depth in bacteria-associated rats [16]. However, No literature reported on the impacts of phytochemicals from *C. cassia* on colonic morphology and microbiota changes.

The human gut is populated by a vast number of bacterial species (more than 800) that reach the highest concentrations in the colon (up to 10^{12} cells per gram faeces) [15]. 98% of these bacteria belonged to phylum *Bacteroidetes* and *Firmicutes* [17]. Moreover, evidence suggested that the relative abundance of the two predominant bacterial divisions differs between lean and obese animals: mice that are genetically obese have fewer amount of *Bacteroidetes* and more *Firmicutes* than their lean siblings [18]. Furthermore, *Bacteroidales* is one of the most dominant groups in *Bacteroidetes*. It has been shown that *Bacteroidales* have significant impacts on human health, involving in carbohydrate fermentation and polysaccharides catabolism [19].

The aim of the study is to investigate the impacts of AEC and EOC of *C. cassia* on rat colonic mucosal morphology and *Bacteroidales* of colon microbiota by morphological observation and terminal restriction fragment length polymorphism (TRFLP) analysis.

2. Experimental

2.1. Preparation of AEC and EOC

C. cassia that obtained from Er Tiantang Co. (Guangzhou, China) were shattered and meshed with 10 meshes. AEC was prepared as follows: *C. cassia* powder was added with 10-fold volumes of distilled water and boiled for 20min at 100 ℃, followed by filtering. The residue was subsequently added with 4-fold volumes of distilled water and continued to be boiled for 20 min at 100 ℃. All of the filtrates was collected and mixed together, concentrated to 2g/mL and then kept at 4 ℃ for further use. EOC was prepared by using hydrodistillation in a Clevenger-type apparatus, according to the method described previously [20] with modification: 50g of cinnamon bark powder was mixed with 200mL of distilled water and then heated at 100 ℃ for 3h. The volatile essential oil was collected and stored at 4 ℃ for further use.

2.2. Animals and Diets

Eight week-old healthy male Sprague-Dawley (SD) rats were obtained from the Animal Center of Southern Medical University (Guangzhou, China). The average weight of rats was 200g ± 20g. After one adaptive week, all animals were randomly classified into 3 groups (10 rats per group): Control group, which was fed with normal diet; AEC group, which was treated with 2mL of aqueous extracts of *C. cassia*, equivalent to about 4.0g raw *C. cassia* bark per kg body weight; EOC group, which was treated with 0.1mL essential oil of *C. cassia*, equivalent to about 0.38g essential oil per kg body weight; The two tested groups were fed with normal diet, too. During the 30 days of feeding, the environment was maintained at a temperature of 23 ± 2 ℃ and relative humidity was 50% to 60%, with a light/dark cycle of 12h. Padding was renewed three times a week, and the rats had free access to food and water during the experiment. The animal experiments were approved by the Department of Care and Use of Laboratory Animal in Jinan University (Guangzhou, China).

2.3. Sampling Collection

After 30 days of feeding, 3 rats were randomly selected from each group and etherized after weighed. The colons of all rats were removed aseptically from the abdominal cavity and dissected free from fat and mesentery. The colon was sampled at 1cm proximal to the ileal valve. Their colonic contents were individually sampled and stored at -20 ℃ for microbiota analysis. Then the colon tissue was fixed immediately with 10% formalin, then embedded with paraffin, sectioned and stained with haematoxylin and eosin for further morphology analysis.

2.4. Colonic Mucosal Analysis

Microstructure analysis was conducted with a light microscope (OLYMPUS X51, Olympus Co., Tokyo, Japan) at 40× and 100× magnification. Computerized morphometric measurements were made of the following: villous height, villous area, mucosal thickness, crypt count, crypt depth, fold thickness were carried out on random and in double blind method by using the image analyzer (DP2-BSW, Olympus Co.).

2.5. Total DNA Extraction and Purification of Colonic Microbe

The bacterial DNA of each sample was extracted by Stool DNA-out kit (AndyBio, Itasca, IL, USA) according to manufacturer's instructions. The total DNA samples were characterized with 1% agarose gel electrophoresis for integrity and size. The DNA was adjusted to 40ng/μL and stored at -20 ℃ before used as templates for PCR.

2.6. TRFLP Analysis

Bacteroidales specific 16S rRNA genes was amplified by the primers 32f (AACGCTAGCTACAGGCTT) which is labeled with FAM, and 708r (CAATCGGAGTTCTTCGTG) [21]. Amplification was performed using an Eppendorf Mastercycler™ thermal cycler (Hamburg, Germany) with the following program: an initial 3 min denaturation at 94 ℃ followed by 30 cycles of 45s denaturation at 94 ℃, 30s annealing at 56 ℃, and 2 min extension at 72 ℃. After the final cycle, 3 min elongating at 72 ℃ completed the PCR. The following reaction cocktail was used: 20ng template DNA; 0.025U/μL Taq DNA polymerase (TaKaRa, Otsu, Shiga, Japan); 10× reaction buffer (Mg^{2+} plus) as supplied by manufacturer; 0.25mM of each primer (synthesized by Shanghai Sangong, China); and 0.2mM of each of the dNTPs (TaKaRa). The reactions were carried out in a final volume of 50μL.

PCR products were further purified by the NewProbe PCR purification kit (New Probe, Beijing, China) using the manufacturer's protocol. The PCR cleanup products were quantified by determining absorption of samples at 260nm. An enzyme digestion was performed on each PCR product using *Hha* I (TaKaRa). Each 40μL digestion used 75ng DNA, 1 U enzyme and 4μL 10× buffer (0.4μL 20μg/mL BSA were added to *Hha* I digestions). Samples were digested for 4h at 37 ℃ and inactivated for 20 min at 65 ℃. TRFLP was performed by Invitrogen (Carlsbad, NM, USA).

2.7. Data Analysis of TRFLP Profiles

Terminal restriction fragments (TRFs) smaller than 50 bp were excluded from the further analysis. Fragments differing by less than 1 bp length were clustered. Relative peak areas of each TRF were determined by dividing the area of the peak of interest by the total area of peaks within the following threshold: a lower threshold at 50 bp and an upper threshold at 600 bp. Only TRFs with relative abundances above 0.5% were included in the remaining analyses. Putative identities for the most dominant peaks were predicted by *in silico* digestion with *Hha* I in the Ribosomal Database Project II (http://rdp.cme.msu.edu) using the TRFLP analysis program TAP.

Replicate profiles from separate DNA extractions and PCR reactions for each sample were compared to identify the subset of reproducible fragment sizes. The average area of each reproducible peak was calculated. The standardized binning criteria used to identify the subset of reproducible peaks were as previously described [22,23]. Ribotype richness (S) was calculated according to the total number of distinct fragments in each sample. The

Shannon diversity index (H) and evenness (E) were calculated according to the methods described previously [23,24]. Jaccard similarity index was calculated using EXCEL to evaluate the similarity for microcosm samples between two groups [25]. Similarity percentage (SIMPER) analysis was further used to identify the fragments that were mainly responsible for the dissimilarity between samples [26,27].

2.8. Statistics

Results are expressed as mean values with their standard deviation (SD). Statistical analyses were conducted with the Statistical Package for Social Science (SPSS for Windows, version 8.0; SPSS Inc., Chicago, IL, USA) to determine if variables differed among treatment groups.

3. Results

There were no mortalities during the experiment and no periods of weight loss or diarrhea observed throughout the study. Moreover, no organ of the rats was found morbid after dissection. The AEC group resulted in less body-weight gain (45.33g, $P<0.01$) after 4 weeks feed intake, the EOC group had no difference in weight gain compared with controls (85.33g vs. 87.67g, $P>0.05$) (Figure 1). Additionally, there was no difference in food intake among groups during the experiments.

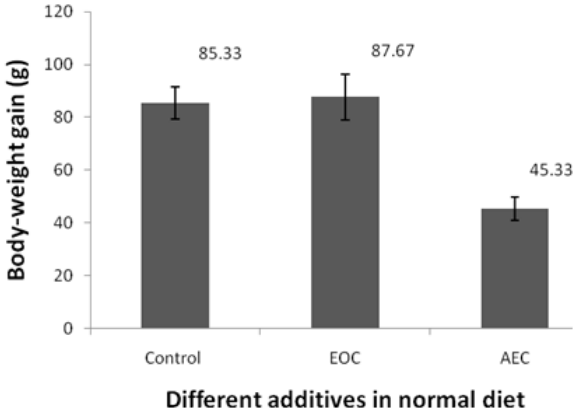

Figure 1. SD rats' body-weight gain of the control, AEC and EOC groups. Values are expressed as means ± SD. ** P<0.01, compared to the control group

Table 1. The colon mucosal morphometry of rat under light microscope at 40 ×magnification

Group	Villous height (μm)	Villous area (μm^2)	Mucosal thickness (μm)	Crypt count (mm^{-2})	Crypt depth (μm)	Fold thickness (μm)
Control	806.38 ±186.12[a]	71107.96 ±31332.35	1057.85 ±217.30	61.43 ±13.43	217.28 ±36.35	215.84 ±25.80
AEC	740.89 ±84.68	49587.00 ±5734.86**	990.72 ±85.01	39.64 ±6.36**	222.70 ±26.45	214.41 ±30.61
EOC	848.28 ±169.47	130839.60 ±63502.51	1225.55 ±234.63	67.14 ±9.29*	295.01 ±103.17*	349.48 ±58.06*

[a] Values are expressed as means ±SD.
* P<0.05, compared to the control group; ** P<0.01, compared to the control group.

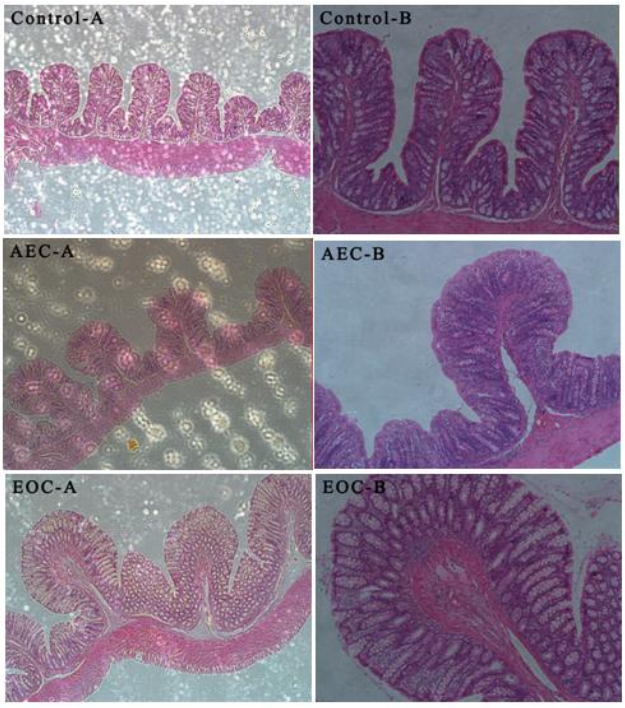

Figure 2. The pictures of rat colonic mucosa. These morphological pictures were captured with the aid of a light microscope. "A" and "B" are used respectively to refer to the pictures amplified 40 × and 100 ×

The morphological pictures of rat colonic mucosa were presented as followed (Figure 2). The crypt count and villous area of the AEC group were significantly lowered ($P<0.01$) than EOC and control groups. The other parameters (e.g. villous height and mucosal thickness) were also reduced with no significance ($P>0.05$). The crypt count, crypt depth and fold thickness in EOC group were increased comparing to control group ($P<0.05$) (Table 1).

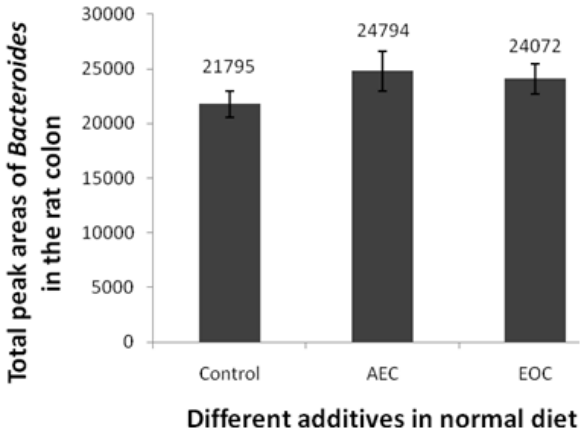

Figure 3. Total peak areas of colonic *Bacteroides* from the control, AEC and EOC groups. Values are expressed as means ± SD. * P<0.05, compared to the control group

After treated with EOC and AEC, both of their total peak areas of *Bacteroidales* significantly increased (P<0.05) (Figure 3). The total average numbers of terminal restriction fragments (*Hha* I digest) in the three groups were all 6. Shannon diversity index and evenness of *Bacteroidales* were individually calculated and presented in Table 2. The value of H and E of the AEC group was significantly higher than those of the control

and EOC group, which showed that the diversity of *Bacteroidales* significantly was improved in the AEC group.

Table 2. Comparison of diversity and evenness indices for the TRFLP profiles (*Hha* I digest) of colonic *Bacteroides* from different groups

Species	Samples	S[a]	H[b]	E[c]
Bacteroides	Control	6	1.747	0.676
	AEC	6	2.585	0.855
	EOC	6	1.741	0.673

[a] Ribotype richness; S = total number of brands in profile.
[b] Shannon diversity index; $H = -\sum (p_i) (\log_2 p_i)$, where p_i is the individual peak area; $H_{max} = \log_2 S$.
[c] Evenness; $E = H / H_{max}$.

The Jaccard index of similarity of *Bacteroidales* was quite low in colon. The similarity of the AEC group was about 32.56%, slightly lower than that of the EOC group (34.68%). The low Jaccard similarity reflected a big individual variation of community structure of *Bacteroidales*.

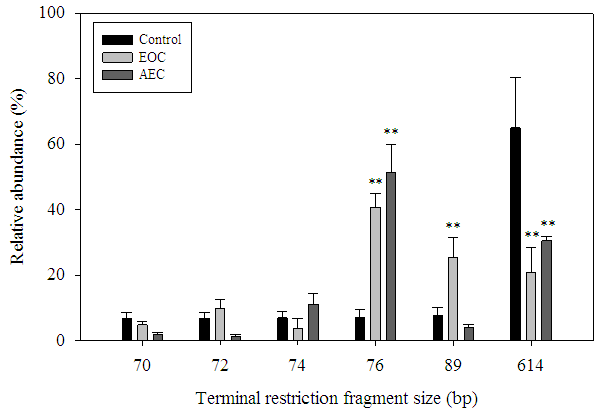

Figure 4. Terminal restriction fragments (*Hha* I digest) and mean relative abundance (%) of colonic *Bacteroides* from the control, AEC and EOC groups. Values are expressed as means ± SD. ** $P<0.01$, compared to the control group

Table 3. Results of SIMPER analysis of TRFLP profiles from different groups

Species	Fragment (bp)	MRA[a] (%)		FC[b] (%)	MRA (%)		FC (%)
		Control	AEC		Control	EOC	
Bacteroides	76	7	51	46	7	41	32
	89	7	4	–[c]	7	25	17
	614	65	30	36	65	21	43

[a] Mean relative abundance of each fragment (*Hha* I digest) as a percentage of total fragment abundance.
[b] Fragment contribution as a dissimilarity percentage between the two groups. Lists are truncated to include only those fragments that contribute no less than 5% to the differences between samples.
[c] Means the fragments contribute less than 5% to the differences between samples.

Rat colons of both the two tested groups had different *Bacteroidales* community structure from those of the control group, according to the TRFLP profiles (*Hha* I digest) of detectable *Bacteroidales* (Figure 4). The SIMPER analysis revealed that the differences of TRFLP profiles (*Hha* I digest) between the control group and the two tested groups were almost driven by the variation in fragments of 76 bp and 614 bp (Table 3). Both EOC and AEC group had a tendency to lower down fragments of 614 bp ($P<0.01$). On the contrary, the EOC and AEC both increased the relative abundance of 76 bp ($P<0.01$). In addition, the fragments of 70 bp, 72 bp, 74 bp and 89 bp were changed by aqueous extracts and essential oil without significant difference ($P>0.05$) (Figure 4).

4. Discussion

The histologic appearance of the colonic mucosa revealed no inflammatory changes such as ulcerations, crypt abscesses, changes in the lamina propia, or alterations in the surface epithelium and crypt epithelium. Morphometric measurements of colonic mucosa (Table 1) showed that AEC diet significantly decreased the measurements of the villous area and the crypt count compared with the controlled.

The rats fed with AEC were prominently leaner than the controlled. This may be explained by the inhibitory effects of phenolic compounds against obesity [28].The bioactive cassia-derived phytochemicals and metabolites could partially accumulate in the contents of rat colon. It has been verified that phytochemicals and their derived products can affect the intestinal ecology as a majority of them can't be fully absorbed and metabolized by the small intestine and the liver, but accumulated in the ileal and colorectal lumen [15]. Therefore, we examined the effects of EOC and AEC on the colonic *Bacteroidales* community.

Lee and Ahn [29] had reported that *C. cassia* bark-derived cinnamaldehyde revealed significant inhibition *in vitro* against specific human intestinal bacteria. However, our study reports the impacts of *C. cassia* extracts on gut microbiota *in vivo*. After 30-day feeding, the amount of colon *Bacteriodales* in both of the test groups increased (Figure 3) and the composition of colon Bacteriodales changed as well (Figure 4). This change may be due to the effects of the phytochemicals and metabolites. Unabsorbed dietary phenolics and their metabolites have been shown to exert varying degrees of bacteriostatic activities on different strains of intestine. Growth of certain pathogenic bacteria such as *Clostridium perfringens*, *Clostridium difficile* and *Bacteroides* spp. was significantly repressed by tea phenolics, while commensal anaerobes like *Clostridium* spp., *Bifidobacterium* spp. and probiotics such as *Lactobacillus* spp. were less severely affected [30].

According to the TRFLP profiles (*Hha* I digest) of detectable *Bacteroidales* (Figure 4), there showed a considerable variation of the terminal restriction fragments among samples. The influence of the two kinds of extracts differed from each other. Two possible explanations can be used to account for this response. Firstly, there do exist a substantial uniqueness in the gut bacterial community among individuals, which has been verified in several others studies [17,18]. We had tried to reduce the impacts came from these individual differences by increasing the repetitions in this study despite it was inevitable. Secondly, the phytochemicals from plant extracts may have inhibitory or promoting effects on different species of bacteria [15]. Given that the components of EOC and AEC differ from each other, it is not hard to understand that one of them would inhibit growth of some species of *Bacteroidales* while stimulate the others. Additionally, the following fragments contributed the main drive to the variation of colon *Bacteroidales*: 76 and 614 bp (Table 3). These fragments deserve to be cloned and identified for

further study. It was somehow regretful that only Hha I was available to hydrolyze the PCR products well when we tried three restriction enzymes (*Hha* I, *Alu* I, and *Hin*d III) to obtain TRFs, which might cause underestimation of microbial diversity.

Finally, the reason we focused on the effects of extracts from *C. cassia* on *Bacteroidales* was because *C. cassia* has been tested for its anti-obesity properties in humans and rats [31,32]. *Bacteroidetes* was also widely reported its association with obesity [17,18]. In our study, the AEC was observed to be functional in upgrading the amount of rat colonic *Bacteroidales*, down-regulating the several parameters of colon mucosa, and decreasing the rats' body-weight gain. This experimental evidence implied that AEC could change both rat colonic mucosa and obesity-associated gut microbiota (*Bacteroidales*). This alteration potentially caused a decreased capacity for energy harvest from the diet as the rats gained less body weight. Despite essential oil also up-regulated the total amount and diversity of colon *Bacteroidales*, its influence on weight gain had scarcely been detected.

In conclusion, the paper reported the effect of AEC and EOC on rat colonic mucosal morphology and *Bacteroidales*. It was found that AEC rather than EOC can change both rat colonic mucosa and probably obesity-associated gut microbiome, which might cause a decreased capacity for energy harvest from the diet, as less body-weight gain was observed in AEC group. Despite essential oil also up-regulated the total amount and diversity of colon *Bacteroidales*, its influence on weight gain had scarcely been detected. However, the abuse of *C. cassia* might have negative effect on human health based on the changes of rat colonic mucosa by the treatment of AEC.

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References

[1] Lee, R. and Balick, M.J."Sweet wood-cinnamon and its importance as a spice and medicine," *Explore*, 1(1). 61-64. Jan. 2005.

[2] Lee, J.S., Jeon, S.M., Park, E.M., Huh, T.L., Kwon, O.S., Lee, M.K. and Choi, M.S. "Cinnamate supplementation enhances hepatic lipid metabolism and antioxidant defense systems in high cholesterol-fed rats, " *Journal of Medicinal Food*, 6(3). 183-191. Fall 2003.

[3] Youn, H.S., Lee, J.K., Choi, Y.J., Saitoh, S.I., Miyake, K., Hwang, D.H. and Lee, J.Y. "Cinnamaldehyde suppresses toll-like receptor 4 activation mediated through the inhibition of receptor oligomerization," *Biochemical Pharmacology*, 75(2). 494-502. Jan. 2008.

[4] Kwon, H.K., Hwang, J.S., So, J.S., Lee, C.G., Sahoo, A., Ryu, J.H., Jeon, W.K., Ko, B.S., Im, C.R., Lee, S.H., Park, Z.Y. and Im, S.H. "Cinnamon extract induces tumor cell death through inhibition of NFκB and AP1," *BMC Cancer*, 10. 392-401. Jul. 2010.

[5] Matan, N., Rimkeeree, H., Mawson, A.J., Chompreeda, P., Haruthaithanasan, V. and Parker, M. "Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions," *International Journal of Food Microbiology*, 107(2). 180-185. Mar. 2006.

[6] Couturier, K., Batandier, C., Awada, M., Hininger-Favier, I., Canini, F., Anderson, R.A.C., Leverve, X. and Roussel, A.M. "Cinnamon improves insulin sensitivity and alters the body composition in an animal model of the metabolic syndrome," *Archives of Biochemistry and Biophysics*, 501(1). 158-161. Sep. 2010.

[7] He, Z.D., Qiao, C.F., Han, Q.B., Cheng, C.L., Xu, H.X., Jiang, R.W., But, P.P.H. and Shaw, P.C. "Authentication and quantitative analysis on the chemical profile of cassia bark (*Cortex Cinnamomi*) by high-pressure liquid chromatography," *Journal of Agricultural and Food Chemistry*, 53(7). 2424-2428. Apr. 2005.

[8] Prasad, K.N., Yang, B., Dong, X.H., et al. "Flavonoid contents and antioxidant activities from *Cinnamomum* species," *Innovative Food Science & Emerging Technologies*, 10(4). 627-632. Apr. 2009.

[9] Kwon, H.K., Jeon, W.K., Hwang, J.S., Lee, C.G., So, J.S., Park, J.A., Ko, B.S. and Im, S.H. "Cinnamon extract suppresses tumor progression by modulating angiogenesis and the effector function of $CD8^+$ T cells," *Cancer Letters*, 278(2). 174-182. Jun. 2009.

[10] Li, L.L. and Yuan, W.J. "Cinnamon oil analysis by GC and GC/MS," *Chinese Journal of Pharmaceutical Analysis*, 1(**2**). 116-118. Feb. 2000.

[11] Shen, Q., Chen, F.L. and Luo, J.B. "Comparison studies on chemical constituents of essential oil from *ramulus cinnamomi* and *cortex cinnamomi* by GC-MS," *Journal of Chinese Medicinal Materials*, 25(4). 257-258. Apr. 2002.

[12] Archer, A.W. "Determination of cinnamaldehyde, coumarin, and cinnamyl alcohol in cinnamon and cassia by high-performance liquid chromatography," *Journal of Chromatography*, 447(1), 272-276. Aug. 1998.

[13] Spencer, J.P.E. "Metabolism of tea flavonoids in the gastrointestinal tract, " *The Journal of Nutrition*, 133(10). 32555-32615.Oct. 2003.

[14] Mattia, A. and Sipes, G.I. *Cinnamyl Alcohol and Related Substances*. WHO Food Additives Series 46. Available: http://www.inchem.org/documents/jecfa/jecmono/v46je07.htm.

[15] Laparra, J.M. and Sanz, Y. "Interactions of gut microbiota with functional food components and nutraceuticals," *Pharmacological Research*, 61(3). 219-225. Mar. 2010.

[16] Kleessen, B., Hartmann, L. and Blaut, M. "Fructans in the diet cause alterations of intestinal mucosal architecture, released mucins and mucosa-associated bifidobacteria in gnotobiotic rats," *British Journal of Nutrition*, 89(5). 597-606. May 2003.

[17] Turnbaugh, P.J., Hamady, M. and Yatsunenko, T. "A core gut microbiome in obese and lean twins," *Nature*, 457(7228). 480-485. Jan. 2009.

[18] Ley, R.E., Turnbaugh, P.J., Klein, S. and Gordon, J.I. Microbial ecology: Human gut microbes associated with obesity. *Nature*, 444(7122). 1022-1023. Dec. 2006.

[19] Li, M., Zhou, H., Hua, W., Wang, B., Wang, S., Zhao, G., Li, L., Zhao, L. and Pang, X. "Molecular diversity of *Bacteroides* spp. in human fecal microbiota as determined by group-specific 16S rRNA gene clone library analysis," *Applied and Environmental Microbiology*, 32(3). 193-200. May 2009.

[20] Demirci, F., Guven, K., Demirci, B., Dadandi, M.Y. and Baser, K.H.C. "Antibacterial activity of two Phlomis essential oils against food pathogens," *Food Control*, 19(12). 1159-1164. Dec. 2008.

[21] Mieszkin, S., Yala, J.F., Joubrel, R. and Gourmelon, M. "Phylogenetic analysis of Bacteroidales16S rRNA gene sequences from human and animal effluents and assessment of ruminant faecal pollution by real-time PCR," *Journal of Applied Microbiology*, 108(3). 974-984. Mar. 2010.

[22] Dunbar, J.M., Ticknor, L.O. and Kuske, C.R. "Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities," *Applied and Environmental Microbiology*, 67(1). 190-197. Jan. 2001.

[23] Mills, D.K., Fitzgeralda, K., Litchfielda, C.D. and Gillevetb, P.M. "A comparison of DNA profiling techniques for monitoring nutrient impact on microbial community composition during

bioremediation of petroleum-contaminated soils," *Journal of Microbiological Method*, 54(1). 57-74. Jul. 2003.

[24] Falk, M.W., Song, K.G., Matiasek, M.G. and Wuertz, S. "Microbial community dynamics in replicate membrane bioreactors-natural reproducible fluctuations," *Water Research*, 43(3), 842-852. Feb. 2009.

[25] Khafipour, E., Li, S., Plaizier, J.C. and Krause, D.O. "Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis," *Applied and Environmental Microbiology*, 75(22), 7115-7124. Nov. 2009.

[26] Clarke, K.R. and Warwick, R.M. "An approach to statistical analysis and interpretation" in *Change in Marine Communities*. 2nd Ed. Plymouth Marine Laboratory, Plymouth, UK. 172.

[27] Rees, G.N., Baldwin, D.S., Watson, G.O., Perryman, S. and Nielsen, D.L. "Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics," *Antonie van Leeuwenhoek*, 86(4). 39-347. Nov. 2004.

[28] Hsu, C.L. and Yen, G.C. "Phenolic compounds: Evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms," *Molecular Nutrition & Food Research*, 52(5). 53-61. May 2007.

[29] Lee, H.S., Ahn, Y.J. "Growth-inhibiting effects of *Cinnamomum cassia* bark-derived materials on human intestinal bacteria," *Journal of Agricultural and Food Chemistry*, 46(1), 8-12. Jan. 1998.

[30] Lee, H.C., Jenner, A.M., Low, C.S. and Lee, Y.K. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Research in Microbiology*, 157(9). 876-884. Nov. 2006.

[31] Solomon, T.P.J. and Blannin, A.K. "Effects of short-term cinnamon ingestion on *in vivo* glucose tolerance," *Diabetes, Obesity and Metabolism*, 9(6). 895-901. Nov. 2007.

[32] Verspohl, E.J., Bauer, K. and Neddermann, E. "Antidiabetic effect of *Cinnamomum cassia* and *Cinnamomum zelanicum in vivo* and *in vitro*," *Phytotherapy Research*, 19(3), 203-206. Mar. 2005.