Original Article

Evaluating the Environmental Health Effect of Bamboo-Derived Volatile Organic Compounds through Analysis the Metabolic Indices of the Disorder Animal Model*

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Abstract

Objective To identify the bamboo VOCs (volatile organic compounds) effect on animal physiological indices, which associated with human health.

Methods GC/MS was used to analyze the volatile organic compounds from Moso bamboo (*Phyllostachys heterocyla cv. pubescens*). The effect of VOCs on environmental health was evaluated by analyzing the metabolic indices of the type 2 diabetic mouse model.

Results Spectra of VOC generated by GC/MS were blasted against an in-house MS library confirming the identification of 33 major components that were manually validated. The relative constituent compounds as a percentage of total VOCs determined were alcohols (34.63%), followed by ether (22.02%), aldehyde (15.84%), ketone (11.47%), ester (4.98%), terpenoid (4.38%), and acids (3.83%). Further experimentation established that the metabolic incidence of the disease can be improved if treated with vanillin, leaf alcohol, β-ionone and methyl salicylate. The effects of these VOCs on type 2 diabetes were evident in the blood lipid and blood glucose levels.

Conclusion Our model suggests that VOCs can potentially control the metabolic indices in type 2 diabetes mice. This experiment data also provides the scientific basis for the comprehensive utilization of ornamental bamboos and some reference for other similar study of environmental plants.

Key words: *Phyllostachys heterocyla cv. pubescens*; Volatile organic compounds; Metabolic index; Type 2 diabetes

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INTRODUCTION

Ornamental plants are used in urban greening, scenic construction and planning within the tourism industry. Of interest is that biologically active compounds are emitted in the odor (volatile organic compounds, VOCs) from certain ornamental plants that are of potential benefit to human health. Recent state-of-the-art plant odor projects were initiated

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with an emphasis on the aesthetic appeal of ornamental plants. Along with in depth research on the collection, separation and identification of plant VOCs in recent years, the concepts such as 'floral therapy', 'balmy therapeutics', and 'forest bath', which were proposed in Europe, America and other countries, have gradually demonstrated their to relevance to human health and well-being[1-3]. Ornamental plant odour studies focus on biological[4], ecological[5], mental and neurological applications[2,6], while studies about its effect on human[7,8] and animal physiological indices[9] are progressing. However, biologically active compounds in plant odors have been demonstrated[10-12].

Bamboo is a gramineous Bambusoideae evergreen perennial plant that is widely regarded as one of the most valuable ornamental plants. It has a high ecological and economic value related to some of its characteristics, which include strong regeneration ability, rapid growth, short foresting period and high yield. In China, the traditional native bamboo industry focused primarily on bamboo wood processing, bamboo shoots and the rapid cultivation of economic species bamboo. In more recent times the ornamental value of a specific cultivar, Tianmushan bamboo, has gained importance. The use of Tianmushan bamboo in landscaping has become an accepted trend and its elevated status promotes the bamboo industry to a higher level of development[13], while fostering a 'green culture' committed to restoring the environment to a more natural state. Ornamental bamboo is prolific, perennially green and has a leaf area index that is larger than the average species, enabling it to release an abundance of odorant VOCs. Following appropriate treatment, Tianmushan bamboo volatiles, like other bamboos, can be used as cosmetics, food additives and air fresheners[14-17].

In this study, VOCs were extracted from the Tianmushan Moso bamboo (*Phyllostachys heterocyla cv*. *Pubescens*) were examined using gas chromatography-mass spectrometry (GC/MS), which is a common method for qualitative analysis of volatiles. The physiological activity of the VOCs that could potentially contribute to human health was investigated using the type 2 diabetic mice model experiment. To our knowledge, a comprehensive investigation that combines the analysis of Tianmushan Moso bamboo VOCs with an animal simulation physiology model has not been reported. The data would provide the scientific basis for the comprehensive utilization of ornamental bamboos as well as a reference for similar studies of ornamental plants. Moreover, understanding the effective components of bamboo organic volatiles and their bioactive functions has important significance for promoting the development of the bamboo industry in the Tianmushan region.

MATERIALS AND METHODS

Materials

Plant Material Moso bamboo leaves were collected from fresh 2-year old plants in January 2014 from the Tianmushan nature reserve, Zhejiang Province (China). Leaves were cleaned, air dried and loaded into a round bottom flask with stopper before being pulped and passed through a 50 mesh sieve.

Animal Material A total of 140 five-week old male ICR mice (International general closed group of mice) with a total body weight ranging from ~25-30 g were used in the experiment. The mice were purchased from the animal center of the Zhejiang Academy of Medical Sciences [License No.: SCXK (Zhejiang) 2008-0033] and maintained under standard laboratory conditions at room temperature (20-25 °C), relative humidity (30%-70%), a 12-h light-dark cycle, with a standard diet and water as desired. The high lipid and sugar feed was purchased from Shanghai Slac Laboratory Animal Co. Ltd. (China), which contained 63% of the standard diet, 16% lard, 10% sucrose, 8% casein, and 3% altodextrin.

Apparatus and Reagents

GC/MS spectra were generated using a gas chromatography-mass spectrometer (7890A GC/5975C MSD, Agilent Technologies, USA). The physiological metabolic indices of the animal model were measured on an ASD-400 semi-automatic biochemical analyzer (Changchun Asia Star Medical Electric Co., Ltd., China). The serum sample was centrifuged at 800 rpm (GuoHua Electric Appliance Co., Ltd, Changzhou China). The temperature of the samples was controlled by placing them in a HH-2 digital constant temperature water bath (GuoHua Electric Appliance Co., Ltd, Changzhou China) and sample weights were determined using an AB104-N electronic analytical balance (1/10000, KenQiang Instruments Co., Ltd, Shanghai China). All pH measurements were determined using a ZD-2 precision acidimeter (Shanghai Lei Ci Device Works,

Shanghai China) with a combined glass calomel electrode. An Eppendorf auto dispenser was used to transfer liquid (KenQiang Instruments Co., Ltd, Shanghai China). Freshly deionized and distilled water (DDW, Sencor-1810D automatic double pure water distillation, Shanghai ShenSheng Technology Co., Ltd., China) was used throughout the experiment. A KQ-250DB ultrasonic cleaner was used to rinse all utensils with DDW (Kunshan Ultrasonic Digitizer Co., Ltd, Shanghai China).

Streptozotocin was purchased from Sigma (≥99%, USA). Vanillin (Va), leaf alcohol (LA), β-ionone (Io) and methyl salicylate (MeS), the assay kit of total cholesterol, triacylglyceride, high density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC) and glucose were all purchased from Beihua Kangtai Clinical Reagent Co., Ltd. (Beijing China). Citric acid, sodium citrate and Tween-80 were purchased from Huadong Medicine Co., Ltd. (Shanghai China). All other reagents were analytical grade.

Methods

VOC Extraction and Analysis The VOCs of bamboo were obtained by extraction and analyzed based on a previously described steam-distillation method[14,17-19]. The 650 mL dH_2O was added to the 2000 mL round bottom flask containing 500 g bamboo leaves. This was heated and distilled by steam. Then 250 mL of distillate was collected and saturated with sodium chloride. The distillate was transferred into a separation funnel and the VOCs extracted by ether, separated and then dried with anhydrous sodium sulfate. This was filtered and the ether was removed using a rotary evaporator. The extraction yield of the bamboo oils from the leaves was about 0.31%. The yellow extract was diluted to 10 mL with ether and stored at 4 °C until analysis by GC/MS.

A direct coupling of GC to electrospray ionization (EI) was used to assay the composition of VOCs extracted from the bamboo. A HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) was used with helium as the carrier gas. Operating conditions were as follows: column temperature: 2 minutes at 60 °C, a gradient of 60-240 °C at 5 °C /min and a final temperature of 2 min at 240 °C. The sample injection (1 μL) was diluted with a split ratio of 100:1. The ion source was EI and the MS operating parameters as follows: ionization potential, 70 eV; voltage multiplier, 1200 V. The detector delay time was set at 5 min after injection.

Type 2 Diabetic Mouse Model Experimental Design A type 2 diabetes mouse model was established by high-lipid diet-ingestion combined with streptozotocin administration[20-21]. After adaptive feeding, the mice were randomly divided into two groups: the control group and glucolipid model group (GMG). After three weeks of adaptation, the mice from the GMG were intravenous injected with streptozotocin (dissolved in pH 4.4 citrate buffer solution and sterilized with 0.45 μm filter membrane attached to an operating ice bath), administered at a dose of 100 mg/(kg·mouse). The animals which exhibited fasting glucose levels equal to or greater than 11.1 mmol/L were screened as type 2 diabetic mice and included in the subsequent experiment (130 GMG mice *vs*. 10 control group mice).

On the basis of GC/MS assay data, the existing conditions of our experiment and associated reagent requisites, vanillin (Va), leaf alcohol (LA), β-ionone (Io) and methyl salicylate (MeS) were selected as the primary VOCs from bamboo in this preliminary study. These four important functional compounds are representative of aldehydic, alcohol, ketone, and ester groups, and can be readily quantified. The selected compounds were prepared as follows[22]: vanillin was diluted to a 0.5% solution with water; the emulsion of leaf alcohol, β-ionone or methyl salicylate were diluted with Tween-80 (ratio of 1:4) and filtered through a 0.45 μm membrane.

All the mice were evenly assigned between fourteen groups according to Table 1 and fed a specified diet for 15 d. The animal experiment conformed to the animal ethical standard of China[23], and all animal treatments were approved by the animal welfare committee of Zhejiang Agricultural & Forestry University.

Biochemical Analysis and the Effect of Individual VOCs on Animal Metabolic Indices After 15 d of treatment, all the mice were given only H_2O for 14 h overnight and then decapitated. The blood was centrifuged for 15 min at 2000 rpm. The supernatant was stored at 4 °C prior to analysis. The blood glucose level was determined using the glucose-oxidase method. Total cholesterol was determined using the CHOD-PAP method. Triacylglyceride was determined with the GPO-PAP method. High density lipoprotein cholesterol (HDLC) was determined with the PTA-Mg method. Low density lipoprotein cholesterol (LDLC) was detected with the PVP method. All the assay procedure following the product manual of the assay kit, and literatures were referenced[24-28].

Data Analysis GC/MS data were analyzed using NIST2008 searching combined with an analytical procedure adapted from a published report[29-31] to determine the relative quantities of VOCs detected (The chemical constituents were analyzed by comparing the obtained spectra data with the standard spectra data, artificial resolving the spectra data and verifying the data documents. The range of mass scan was 12-4000 amu and the mass fraction of each compound was calculated by peak area normalization method). Software SPSS 13.0 was used to compute the comparative influence of VOCs on the physiological metabolic indices of the mice. Results were reported as the mean±standard error of the mean. Differences in metabolic responses were determined using an analysis of variance (ANOVA). Statistical significance was set at $P<0.05$.

RESULTS

GC/MS Analysis of VOCs

The main compound detected by GC-MS was leaf alcohol, which accounted for 65% of the total VOCs detected. Other compounds were present at very low levels (Figure 1A, Table 2). The leaf alcohol eluted very early (4.5 min) and as such the detector delay time was extended from 4 min to 5 min to remove this peak from detection, thereby enhancing the relative areas of the other peaks (Figure 1B, Table 3)[32].

Table 1. Type 2 Diabetic Mouse Model Experimental Design

Group Number	Diet Content	Dosage Type (low, or high)	Dosage Compound	Dosage Compound Concentration (mg/kg, i.g.)
1	standard diet	-	distilled water	-
2	high lipid and sugar diet	-	distilled water	-
3	high lipid and sugar diet	high	vanillin	50.0
4	high lipid and sugar diet	intermediate	vanillin	25.0
5	high lipid and sugar diet	low	vanillin	12.5
6	high lipid and sugar diet	high	leaf alcohol	8.48
7	high lipid and sugar diet	intermediate	leaf alcohol	4.24
8	high lipid and sugar diet	low	leaf alcohol	2.12
9	high lipid and sugar diet	high	β-ionone	9.45
10	high lipid and sugar diet	intermediate	β-ionone	4.73
11	high lipid and sugar diet	low	β-ionone	2.36
12	high lipid and sugar diet	high	methyl salicylate	11.83
13	high lipid and sugar diet	intermediate	methyl salicylate	5.91
14	high lipid and sugar diet	low	methyl salicylate	2.96

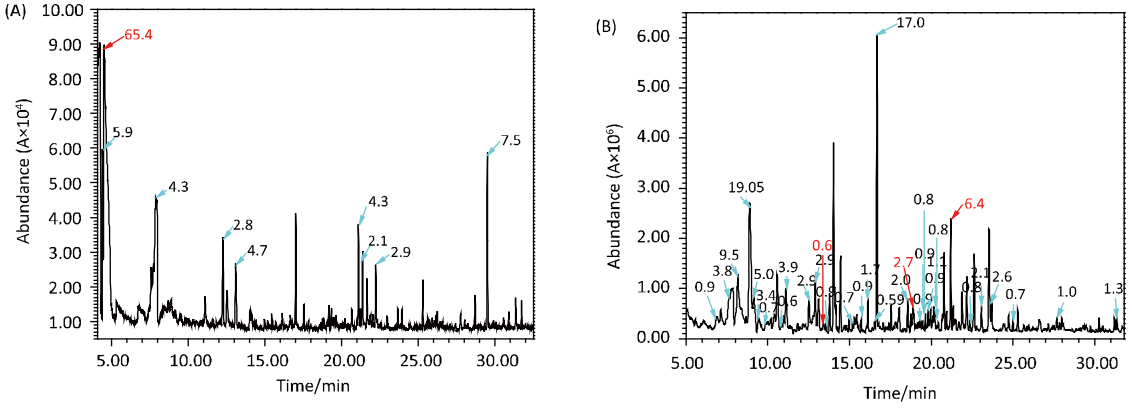

Figure 1. TIC-trace of VOCs for *Phyllostachys heterocyla cv. pubescens* (A. the solvent delay time=4 min; B. the solvent delay time=5 min).

A total of 33 components were identified and manually validated. The largest peak resulting from the bamboo VOC extract was benzyl alcohol, which reaches to 19.0%. Table 3 also shows that the influence of leaf alcohol has been avoided by using the method of delay time (the retention time of leaf

Table 2. The Analysis of VOCs for *Phyllostachys Heterocyla cv. Pubescens* (the solvent delay time=4 min)

No.	Compound	Molecular Formula	Molecular Weight	Retention Time (min)	Relative Content (%)
1	trans-3-hexen-1-ol	$C_6H_{12}O$	100	4.4	5.9
2	Leaf alcohol	$C_6H_{12}O$	100	4.5	65.4
3	2,4-dimethyl-3-hexanol	$C_8H_{18}O$	130	7.6	4.3
4	2,5-dimethyl-benzaldehyde	$C_9H_{10}O$	134	12.5	2.8
5	Azulene	$C_{10}H_8$	128	13.1	4.7
6	α-ionone	$C_{13}H_{20}O$	192	21.1	4.3
7	Cashmeran	$C_{14}H_{22}O$	206	21.6	2.1
8	Dihydroactinidiolide	$C_{11}H_{16}O_2$	180	22.2	2.9
9	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	29.5	7.5

Table 3. The Analysis of VOCs for *Phyllostachys Heterocyla cv. Pubescens* (the solvent delay time=5 min)

No.	Compound	Molecular Formula	Molecular Weight	Retention Time (min)	Relative Content (%)
1	Benzaldehyde	C_7H_6O	106	6.9	0.9
2	Hexanoic acid	$C_6H_{12}O_2$	116	7.6	3.8
3	(Z)-5-octen-1-ol	$C_8H_{16}O$	128	8.2	9.5
4	Benzyl alcohol	C_7H_8O	108	8.9	19.05
5	Benzeneacetal dehyde	C_8H_8O	120	9.1	5.0
6	Decalactone	$C_{10}H_{18}O_2$	170	9.4	3. 4
7	Citronellal	$C_{10}H_{18}O$	154	9.9	0.7
8	Undecanal	$C_{11}H_{22}O$	170	10.8	0.6
9	Phenylethyl alcohol	$C_8H_{10}O$	122	11.1	3.9
10	4-ethyl-benzaldehyde	$C_9H_{10}O$	134	12.5	2.9
11	Rose oxide	$C_{10}H_{18}O$	154	12.9	2.9
12	Methyl salicylate	$C_8H_8O_3$	152	13.4	0.6
13	Safranal	$C_{10}H_{14}O$	150	13.6	0.9
14	Widdrol	$C_{15}H_{26}O$	222	15.2	0.7
15	Farnesol	$C_{15}H_{26}O$	222	15.7	0.9
16	Indole	C_8H_7N	117	16.1	1.7
17	Cinerolone	$C_{10}H_{14}O_2$	166	16.5	0.59
18	p-acetylanisole	$C_9H_{10}O_2$	150	16.7	17.0
19	Damascenone	$C_{13}H_{18}O$	190	18.6	2.0
20	Vanillin	$C_8H_8O_3$	152	18.9	2.7
21	α-funebrene	$C_{15}H_{24}$	204	19.3	0.9
22	α-ionone	$C_{13}H_{20}O$	192	19.6	0.9
23	Cumaldehyde	$C_{10}H_{12}O$	148	19.8	0.8
24	Caryophyllene oxide	$C_{15}H_{24}O$	220	20.0	0.9
25	Isoeugenol	$C_{10}H_{12}O_2$	164	20.2	1.1
26	trans-geranylacetone	$C_{13}H_{22}O$	194	20.2	0.8
27	β-ionone	$C_{13}H_{20}O$	192	21.2	6.4
28	Neocurdione	$C_{15}H_{24}O_2$	236	22.4	0.8
29	Eugenol methyl ether	$C_{11}H_{14}O_2$	178	23.1	2.1
30	Neoclovene	$C_{15}H_{24}$	204	23.7	2.6
31	Cedrol	$C_{15}H_{26}O$	222	25.0	0.7
32	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	27.7	1.0
33	Octadecanal	$C_{18}H_{36}O$	268	31.3	1.3

alcohol is less than 5 min). Among 33 main components, alcohols compounds account for 35%, followed by ether 22%, aldehyde 16%, ketone 11%, ester 5%, terpenoid 4%, and acids 4% in relatives' terms. The single components of vanillin, leaf alcohol, β-ionone and methyl salicylate were selected as the target compounds of VOCs of bamboo to perform further research.

According to some references, GC-MS is used as a common method to qualitative analyzes the contents of volatiles, and the qualitative result is limited, such as references[14]. And the volatile compositions content from different bamboos and different regions is different, so there are some differences between our results and that in references which are maintained to research in further research.

Establishing a High Glucolipid Mice Model

The results for the control group and the mice fed a modified diet to establish a model group of high glucolipid mice are shown in Table 4. The glucose, triacylglyceride, total cholesterol, LDLC in high glucolipid model mice increased significantly compared to the control mice (P<0.01), whereas the content of HDLC decreased significantly compared to control mice (P<0.01). Therefore, streptozotocin-induced type 2 diabete was successfully modeled in the mice treated in this study (Figure 2).

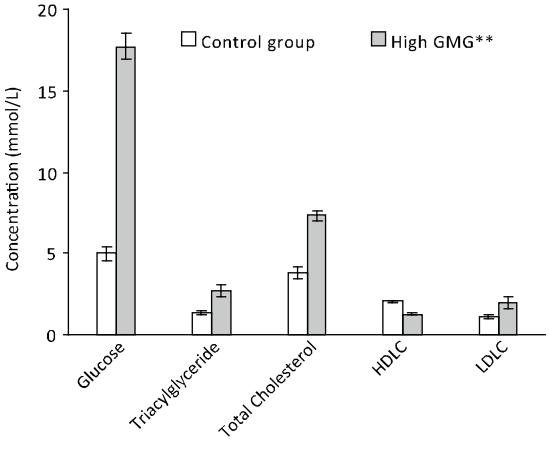

Figure 2. The metabolic indexes of blood glucose and blood lipid of the control group and the high GMG mice. *: P<0.05, **: P<0.01 (Compared with the normal group).

Table 4. All Data for the Effect of the VOCs on the Blood Glucose and Blood Lipid of Type 2 Diabetic Mice

Group	*n*	Glucose (mmol/L)	Triacyl Glyceride (mmol/L)	Total Cholesterol (mmol/L)	HDLC (mmol/L)	LDLC (mmol/L)
Control group	10	4.97±0.43	1.37±0.16	3.78±0.36	2.07±0.07	1.12±0.11
High GMG	10	17.69±0.79**	2.68±0.39**	7.32±0.29**	1.27±0.09**	1.95±0.35**
High dose of vanillin	10	11.38±0.59$^{\#\#}$	1.30±0.07$^{\#\#}$	3.34±0.18$^{\#\#}$	1.72±0.08$^{\#\#}$	1.80±0.24
Middle dose of vanillin	10	13.23±0.49$^{\#\#}$	1.43±0.15$^{\#\#}$	4.85±0.50$^{\#\#}$	2.02±0.34$^{\#\#}$	2.11±0.36
Low dose of vanillin	10	13.32±1.31$^{\#\#}$	1.63±0.12$^{\#\#}$	5.44±0.50$^{\#\#}$	2.31±0.31$^{\#\#}$	2.11±0.42
High dose of leaf alcohol	10	12.65±0.59$^{\#\#}$	1.69±0.33$^{\#\#}$	5.22±0.19$^{\#\#}$	1.94±0.27$^{\#\#}$	1.31±0.40$^{\#\#}$
Middle dose of leaf alcohol	10	13.79±0.74$^{\#\#}$	1.91±0.20$^{\#\#}$	5.73±0.32$^{\#\#}$	2.12±0.23$^{\#\#}$	3.11±0.17$^{\#\#}$
Low dose of leaf alcohol	10	15.92±0.72$^{\#\#}$	1.14±0.08$^{\#\#}$	4.35±0.23$^{\#\#}$	1.58±0.14$^{\#\#}$	2.90±0.22$^{\#\#}$
High dose of β-ionone	10	11.82±0.29$^{\#\#}$	1.41±0.13$^{\#\#}$	4.50±0.33$^{\#\#}$	1.46±0.16	2.34±0.31$^{\#}$
Middle dose of β-ionone	10	10.20±0.57$^{\#\#}$	1.39±0.09$^{\#\#}$	4.08±0.25$^{\#\#}$	1.49±0.17$^{\#}$	1.83±0.57
Low dose of β-ionone	10	14.84±0.57$^{\#\#}$	1.51±0.10$^{\#\#}$	5.46±0.12$^{\#\#}$	1.66±0.33$^{\#\#}$	2.80±0.42$^{\#\#}$
High dose of methyl salicylate	10	11.96±0.39$^{\#\#}$	1.26±0.12$^{\#\#}$	4.48±0.38$^{\#\#}$	1.59±0.19$^{\#\#}$	1.98±0.42
Middle dose of methyl salicylate	10	10.66±0.68$^{\#\#}$	1.00±0.13$^{\#\#}$	3.96±0.28$^{\#\#}$	1.49±0.18$^{\#}$	1.78±0.45
Low dose of methyl salicylate	10	13.09±0.44$^{\#\#}$	1.90±0.15$^{\#\#}$	4.95±0.32$^{\#\#}$	1.45±0.35	2.37±0.22$^{\#\#}$

Note. $^{*}P$<0.05, $^{**}P$<0.01 (Compared with the normal group); $^{\#}P$<0.05, $^{\#\#}P$<0.01 (Compared with the high glucolipid model group).

Effect of Vanillin on the Metabolic Indices of Type 2 Diabetic Mice

The effect of vanillin on blood glucose and blood lipids of type 2 diabetic mice is presented in Figure 3A. The metabolic indices of glucose, triacylglyceride and total cholesterol all significantly decreased compared to the high glucolipid group (P<0.01). Comparatively, the high dose group had the most significant decrease. The metabolic indices of HDLC increased significantly for the high glucolipid group (P<0.01) compared to the group dosed with vanillin, but the low dose group is the most significant to increase the HDLC value. There is no statistical significance on LDLC of each dose group (P>0.05) with vanillin treated. Overall, the effect of a high dose of vanillin on blood glucose and blood lipid metabolic indices proved to be the most beneficial dose to treat type 2 diabetes in mice. Triacylglyceride, and total cholesterol decreased to control group levels, while blood glucose decreased to a level that was borderline diabetic (11.1 mmol/L). Although HDLC increased to levels comparable to the control group, the LDLC was unaffected following the high vanillin treatment. Therefore, the high dose of vanillin proved to be the most appropriate dose of all dosages tested.

Effect of Leaf Alcohol on the Metabolic Indices of Type 2 Diabetic Mice

The effect of leaf alcohol on the blood glucose and blood lipid metabolic indices of type 2 diabetic mice is presented in Figure 3B. Glucose, triacylglyceride and total cholesterol were significantly lower than that of the high glucolipid group (P<0.01) with each dose group. However, only the triacylglyceride (low dose) was within the control group value. The blood HDLC concentration was significantly higher than that of the high glucolipid group (P<0.01) with each dose group, with the high and intermediate leaf alcohol doses producing concentrations equivalent to the control group. Only the high dose of leaf alcohol improved LDLC concentration (P<0.01), but this was to the level of the control group. In summary, the high dose of leaf alcohol proved to be the most beneficial dosage for this group.

Effect of β-ionone on the Metabolic Indices of Type 2 Diabetic Mice

The effect of β-ionone on the blood glucose and blood lipid metabolic indices of type 2 diabetic mice is presented in Figure 3C. Metabolic indices of glucose, triacylglyceride and total cholesterol were significantly decreased compared to high glucolipid

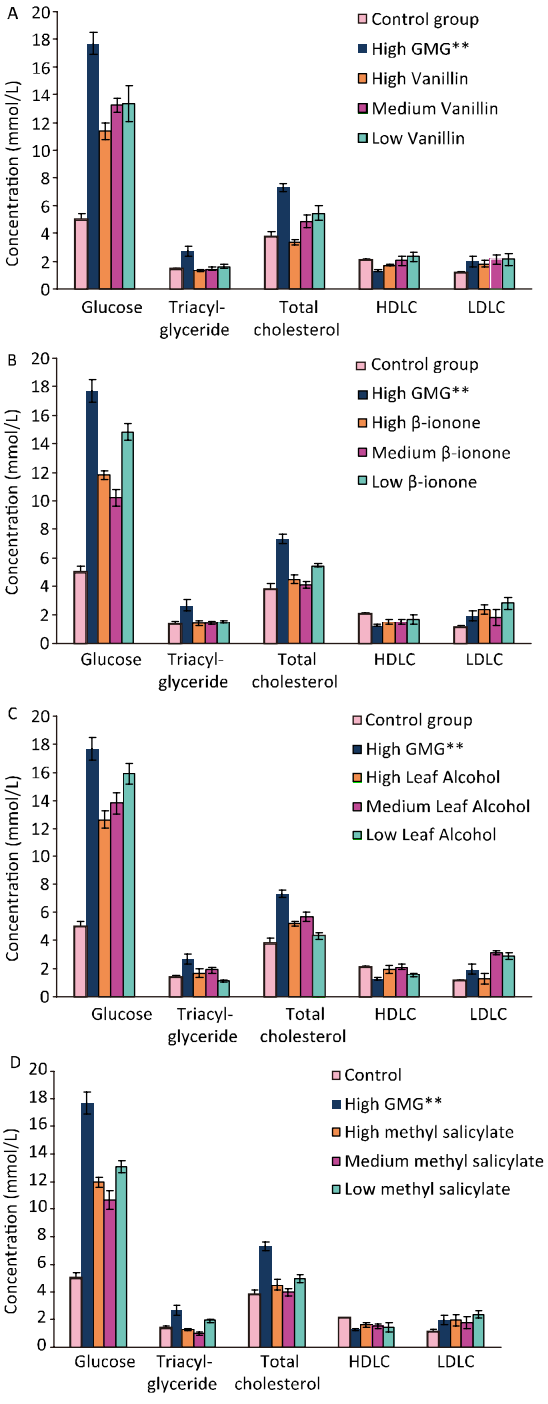

Figure 3. The effect of the four VOCs on blood glucose and blood lipid metabolism indexes of type 2 diabetic mice (A, B, C, D represent Va, LA, β-ionone and the methyl salicylate, respectively.) *: P<0.05, **: P<0.01 (Compared with the normal group).

group (P<0.01) for all dose strengths. The medium dose group displayed the best response (Figure 3C). Data for metabolic indices of HDLC and LDLC were very similar to the high glucolipid model group values, indicating little to zero significant improvement for β-ionone supplementation.

To summarize all the results of each dose group, the medium β-ionone dose had the most bneficial effect on blood glucose, triacylglyceride and blood lipids for type 2 diabetic mice.

Effect of the Methyl Salicylate on the Metabolic Indices of Type 2 Diabetic Mice

The effect of methyl salicylate on the blood glucose and blood lipid metabolic indices of type 2 diabetic mice is displayed in Figure 3D. The results were similar to those of the previous two compounds: the metabolic indices for glucose, triacylglyceride and total cholesterol were improved, while HDLC and LDLC were not improved relative to the high glucolipid model group.

The effect of medium dose of methyl salicylate on blood glucose and blood lipid metabolic indices of type 2 diabetic mice was the most beneficial dose strength. The contents of glucose, triacylglyceride and total cholesterol with medium strength dose of methyl salicylate were significantly decreased, and the content of HDLC is significantly increased, thereby improving the metabolic indices for type 2 diabetic mice.

The Comprehensive Analysis of Effect of the Typical VOCs on Blood Glucose and Blood Lipid Metabolic Indices of Type 2 Diabetic Mice

The effect of four typical VOCs of bamboo on blood glucose and blood lipid metabolic indices of type 2 diabetic mice is displayed in Table 5 and Figure 4 such it is easy to see which compounds and doses achieved effects similar to the values for the control group. It was clear that the three concentrations of the four VOCs improved blood glucose and blood lipid metabolic indices of type 2 diabetic mice to varying extents. The greatest glucose lowering effect was attained using β-ionone at the medium-range dose; methyl salicylate at the medium-range dose best lowered triacylglyceride; while the best total cholesterol lowering effect was attained using vanillin at the highest dose tested. A low dose of vanillin had the best effect on increasing HDLC, while leaf alcohol at the highest dose had the best effect on lowering LDLC.

According to Table 5 and Figure 4, it can be inferred that different VOCs of bamboo influence different metabolic indices of animals. The concentration should be set at a concentration limit, while the benefit of using two or more volatile components and assessing their synergistic role in promoting optimal health should still be investigated.

Table 5. The Effect of Four Typical VOCs of Bamboo on Metabolic Indices of Type 2 Diabetic Mice

Group	Blood Glucose	Total Cholesterol	Triacyl Glyceride	HDLC	LDLC
High vanillin	BD	Yes	Yes	No	No
Medium vanillin	No	No	Yes	Yes	No
Low vanillin	No	No	No	Yes	No
High leaf alcohol	No	No	No	Yes	Yes
Medium leaf alcohol	No	No	No	Yes	No
Low leaf alcohol	No	No	Yes	No	No
High β-ionone	BD	No	Yes	No	No
Medium β-ionone	BD	Yes	Yes	No	Yes
Low β-ionone	No	No	Yes	No	No
High methyl salicylate	No	No	Yes	No	No
Medium methyl salicylate	BD	Yes	Yes	No	No
Low methyl salicylate	No	No	No	No	No

Note. Yes: lowered to within the control group range; No: did not lower to within the control group range; BD: borderline-lowered to within or below the type 2 diabetic level (11.1 mmol/L).

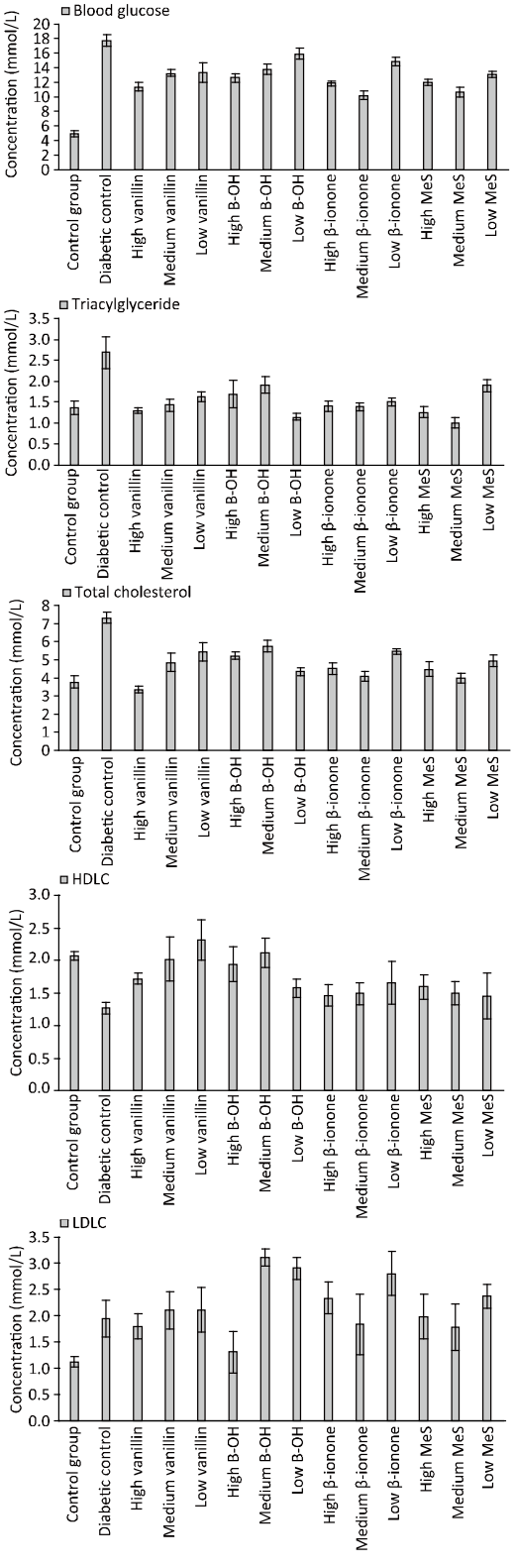

Figure 4. The four VOCs on the blood glucose and blood lipid metabolism indexes of type 2 diabetic mice.

DISCUSSION

GC/MS Analysis of Bamboo VOCs

The VOC detected at a retention time of 4 min was leaf alcohol. Other VOCs were lower present at detectable levels. Although the amount of leaf alcohol determined in this study was higher than that previously reported on Moso bamboo VOCs[16-17,33], it corroborates previous studies where leaf alcohol was the main detectable component of the VOCs. The difference concentrations reported is likely due to the growth of bamboo in different regions and climates[34]. As the retention time of leaf alcohol was ~4.5 min, the detector delay time was set to 5 min. This allowed easier visualization of other VOCs, as the peak sizes were not lowered relative to the leaf alcohol peak. This result could provide a reference that a different delay time is required to detect the VOCs by using GC/MS in the future work.

The Influence of Selected Compounds and their Dose Strength on the Physiological Metabolism of Type 2 Diabetic Model Mice

Typically, the ideal physiological effect of VOCs on the type 2 diabetes model mice would be a lower level of glucose, triacylglyceride, total cholesterol and LDLC, and an increase in HDLC levels. The analysis of the physiological aspects of the index (organs, e.g., liver and kidneys) were not considered in this preliminary study. Although we found that Some treatment of the VOCs was lower levels of glucose, triacylglyceride, total cholesterol, LDLC, and increased the HDLC concentration, while a single VOC did not manage to improve all the metabolic indices to within the control group range.

With regard to blood glucose levels, it was evident that all compounds improved the glucose levels relative to the diabetic control group. However, none of the improvements were to an extent that brought the levels back to with the control group value for this metabolic indices. At best, the levels were lower such that they fell to the level of borderline diabetic (equal or lower than 11.1 mmol/L). These levels were attained by all of the mice receiving a medium β-ionone dose, most of the mice receiving a medium methyl salicylate dose and a few of the mice receiving the high vanillin dose.

A high vanillin and a medium methyl salicylate or β-ionone dose improved the total cholesterol levels to that of the control group. This was also

significantly lower than the diabetic control group values for all four compounds at all doses.

The treatments were all particularly effective at lowering the triacylglyceride levels to within the levels of the control group; and all treatments were significantly lower than the diabetic control group. All four VOCs lowered the levels to control group levels at one or more concentrations tested. In particular, β-ionone achieved control group levels even at the lowest concentration assessed.

The HDLC levels were all within the control group levels after treatment with medium or low doses of vanillin and high or medium does of β-ionone. All treatments improved the HDLC levels relative to the diabetic control group, but the only treatment that did not improve the HDLC levels significantly above that of the diabetic control group was the low dose of methyl salicylate.

However, only some of the high leaf alcohol-dosed mice attained acceptable control group LDLC concentrations. Among the treatment groups, leaf alcohol at a medium and low dose, as well as β-ionone at a high dose, significantly increased (P<0.01); LDLC levels compared to the mice in the type 2 diabetic group. This indicated that the two lower doses were actually detrimental as they increased the metabolic indices to a greater extent than the high GMG diet alone.

An integrated analysis of these metabolic indices of all treatment groups with specific volatile component doses showed that each volatile component treatment group possessed an optimal concentration with the most benficial effect of the metabolic indices on type 2 diabetic mice. The data suggests that the optimal concentration for vanillin treatment and leaf alcohol was at the higher doses at 50 and 8.48 mg/kg, respectively, while β-ionone and methyl salicylate were better at the medium doses at 9.45 and 11.83 mg/kg, respectively. According to the experimental results, we believe that the influence of VOCs on the physiological metabolism of animals was not only concentration dependent. The ingestion, acid or base hydrolysis, gut-associated metabolic conversion and crossover into the blood stream are complex processes. This was illustrated by the varying effect of the different doses of the VOCs assessed. The utilization of animal experiments to model the effects of volatile components should consider metabolism as a complex process. The overall effect of the volatile component should be considered, and it is inaccurate that more biologically active the volatile component is, the better the effect on metabolic indices. This study was only conducted using a single component at one time; it is distinctly likely that combinations of the compounds could have synergistic affects, thereby lowering the concentration required for an optimal effect and also attaining desirable control group values for all the metabolic indices in a single treatment.

CONCLUSIONS

The relationship between aromatic plants volatile organic compounds (VOCs) and human physiological metabolic indices has not been studied systematically and effectively[35]. Little is known about the effect of aromatic plants' VOCs[36], and to our knowledge, this is the first report of the physiological effect of bamboo VOCs on metabolism. In this study, VOCs from disease-free bamboo plants (2 to 3 years old) lowered all of the metabolic indices significantly. Levels equal to the control group were attained for total cholesterol, triacylglyceride, high density lipoprotein cholesterol and low density lipoprotein cholesterol. Although blood glucose was not lowered to that of the control group, it was significantly lowered and some doses attained levels below the type 2 diabetic threshold of 11.1 mmol/L. Although the pharmacokinetics of single component VOCs is reported here, the integral bioactivity of multicomponent testing requires further studies. Additionally, the impact of culture conditions and bamboo plant health requires further study, as these may affect the VOC composition and abundance - and elicit different effects on the metabolism of mice.

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