Identification of Volatile Components of Fresh *Indocalamus Latifolius* Leaves by HS-SPME-GC-MS

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Abstract: The volatile components of fresh *Indocalamus latifolius* leaves were determined. They will provide theoretical basis to develop and utilize the value of *Indocalamus latifolius* leaves. Headspace solid-phase microextraction method (HS-SPME) with gas chromatography-mass spectrometry (GC-MS) was used for the analyzing of volatile components from the fresh *Indocalamus latifolius* leaves. The relative percentage proportions of volatile substances in sample were determined and compared. A total of 38 volatile components were tentatively identified from fresh *Indocalamus latifolius* leaves by GC-MS, and the most abundant compounds were cis-3-hexenol (33.893%), (E)-2-hexenal(20.461%), 1-hexanol (9.159%), 2-ethylfuran (4.923%), 1-penten-3-ol (2.224%) and hexanal(2.207%). The GC-MS method had the rapid, simple and accurate advantages for the studying of the volatile components in fresh *Indocalamus latifolius* leaves. It provides the basis for the further studies on the volatile components of *Indocalamus latifolius* leaves.

1 INTRODUCTION

It is a traditional Chinese custom to eat rice dumpling on Dragon Boat Festival in China for thousands of years, and rice dumpling is also a kind of festival food which is popular with the public. A wide variety of rice dumpling leaves are the indispensable materials for making this festival food, and the south of China is generally dominated by leaves of *Indocalamus* and *Phrynium capitatum* Willd, while in the north of China the reed leaves are used to make rice dumpling (Cen et al., 2017; Wang, 2016). Among them, the leaves of *Indocalamus* have been sourced for making rice dumpling because it has wide and big leaf blade, as well as its special scent. In addition, it has antiseptic and sterilization effects (Xu and Xu, 1991). The leaves of *Indocalamus* can be divided into *Indocalamus latifolius* leaves, *Indocalamus tessellatus* leaves, *Indocalamus emeiensis* leaves and other 24 different varieties (Ai et al., 2004). Shuifang Li et al demonstrated that its aroma was associated with the volatile components in fresh *Indocalamus emeiensis* and *Indocalamus tessellatus* leaves, and the antiseptic effects of *Phrynium capitatum* Willd was mainly associated with the volatile components with antioxidant and bacteriostatic activity (Yu et al., 2002; Li et al., 2015; Lai et al., 2013). The *Indocalamus latifolius* leaves is one of the most common materials for making rice dumpling, and only the volatile components of the dry *Indocalamus latifolius* leaves were identified (Li et al., 2007a; Li et al., 2007b). However, the fresh leaves are often used to wrap rice dumpling. To our knowledge, there is no relevant reports on the volatile components of fresh *Indocalamus latifolius* leaves. Therefore, GC-MS was used to analyze the volatile components of fresh *Indocalamus latifolius* leaves in this study and the results provided scientific basis for elucidating the chemical substance base of the volatile components of fresh *Indocalamus latifolius* leaves. It will provide theoretical basis and technology indorsation for the further promotion of *Indocalamus latifolius* leaves.

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2 EXPERIMENRAL

2.1 Materials

The fresh leaves of *Indocalamus latifolius* were purchased from Beijing Hualian Supermarket in Guiyang City, Guizhou Province, and has been identified as *Indocalamus latifolius* by Professor Xiangpei Wang of Guiyang University of Traditional Chinese Medicine. The fresh leaves were cut into small pieces, they were put in storage bags, and the bags were kept in the refrigerator at -20℃.

2.2 Solid-phase Micro Extraction Procedure

The sample was accurately weighed (0.5g) and placed into 25 ml sample vial. Then, a 2cm-50/30um DVB/CAR/PDMS Stable Flex fiber(American Supelco company) was used to the headspace above the sample for extracting 45 min under about 80℃. The extraction head was removed from sample vial and immediately inserted onto the GC injection port,the sample thermal desorption for 3 min and then directly injected into GC.

2.3 Gas Chromatography–Mass Spectrometry Analysis

The analyses of gas chromatography was performed on an HP6890/5975C (Agilent USA) using a ZB-5MSi (5% phenyl-95% dimethylpolysiloxane) fused silica capillary column, The pre-column pressure was 7.62psi. The GC oven temperature was programmed to hold at 40℃ for 2 min and then to increase to 260℃ at 5℃ / min, running 46min. The injector temperature were set at 250℃. High purity helium (purity 99.999%) was used as carrier gas with a flow rate of 1.0ml / min, samples were injected in splitless mode. The solvent delay was 1min.

The Aglient 5975C mass spectrometer was operated in the electron impact (EI) mode using an ionisation energy at 70eV with a quadrupole temperature set of 150℃ and a ionisation source temperature of 230℃. The multiplier voltage was 1671V, the interface temperature was 280℃, the emission current was 34.6μA and mass range was 29-500 amu.

2.4 Data Analysis

The peaks in the total ion flow map were retrieved by the mass spectrometer computer data system and identified by comparison to reference mass spectra in the Nist2005 and Wiley275 databases. The instrument Chemstation data processing system was used to determine the relative concentrations of the analytes by the peak area normalization method.

3 RESULTS

The volatile components were isolated and identified(Table 1). The total ion chromatogram of fresh *Indocalamus latifolius* leaves is shown in Figure 1, and the corresponding volatile compounds are listed in Table 1. A total of 38 volatile components were tentatively identified in fresh *Indocalamus latifolius* leaves by GC-MS, including 8 alcohols compounds (47.453%), 11 aldehydes (28.202%), 7 terpenes (12.072%), 7 hydrocarbons (0.692%), esters only detected cis-3-hexenyl acetate (0.456%), heterocyclic detected 2-ethylfuran (4.923%) and 2-amylfuran (0.102%), aromatic detected benzaldehyde (1.593%) and benzeneacetaldehyde (0.925%), alcohol components mainly cis-3-hexenol (33.893%) and 1-hexanol (9.159%), aldehyde components is dominated by (E)-2-hexenal (20.461%), terpene component mainly neophytadiene (9.463%), and the relative percentage proportions of hydrocarbons were low.

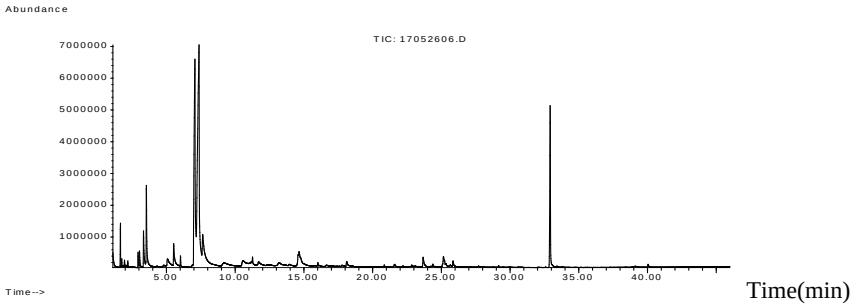

Figure 1: TIC of volatile components extracted from fresh *Indocalamus latifolius* leaves.

Table 1: Percentages of volatile components extracted from fresh *Indocalamus latifolius* leaves.

No.	Retention time	Volatlie components	Relative percentage proportions (%)
1	1.75	ethanal	0.19
2	1.86	ethanol	0.04
3	2.08	1,3-pentadiene	0.02
4	2.19	isobutanal	0.21
5	2.42	butanal	0.04
6	2.72	isobutyl alcohol	0.01
7	2.94	3-methylbutanal	0.63
8	3.05	2-methylbutanal	0.89
9	3.33	1-penten-3-ol	2.22
10	3.54	2-ethylfuran	4.92
11	4.76	(E)-2-pentenal	0.04
12	5.00	1-pentanol	0.01
13	5.07	(Z)-2-pentenol	1.37
14	5.50	hexanal	2.20
15	7.07	(E)-2-hexenal	20.5
16	7.37	cis-3-hexenol	33.9
17	7.64	1-hexanol	9.15
18	9.19	(E,E)-2,4-hexadienal	1.63
19	10.58	benzaldehyde	1.59
20	11.19	2-amylfuran	0.10
21	11.7	cis-3-hexenyl acetate	0.45
22	12.47	(E,E)-2,4-heptadienal	0.29
23	13.15	benzeneacetaldehyde	0.92
24	14.47	linalool	0.73
25	14.65	nonanal	1.57
26	17.42	dodecane	0.01
27	17.77	2,6-dimethylundecane	0.15
28	18.00	beta.-cyclocitral	0.61
29	22.21	farnesane	0.06
30	22.85	tetradecane	0.14
31	23.67	alpha.-ionone	0.87
32	25.08	trans-.beta.-farnesene	0.06
33	25.15	beta.-ionone	0.85
34	25.33	pentadecane	0.22
35	25.65	E,E-.alpha.-farnesene	0.13
36	27.71	hexadecane	0.11
37	29.96	heptadecane	0.02
38	32.92	neophytadiene	9.46

4 CONCLUSIONS AND DISCUSSION

The experimental results show that the main volatile components of fresh *Indocalamus latifolius* leaves were aromatic, heterocyclic, aldehydes, alcohols and terpenes, among them, the relative percentage proportions of cis-3-hexenol and (E)-2-hexenal were the most abundant, As we know, cis-3-hexenol mainly confers the aroma of herbs and green leaves, while (E) -2-hexenol mainly exert an aroma of fresh fruit (Li, 2007). These ingredients may play an important role in the overall aroma of fresh *Indocalamus latifolius* leaves. The relative percentage proportions of alcohols, aldehydes and terpenes were the greatest among the volatile components of fresh *Indocalamus latifolius* leaves, and (E)-2-hexenal resulted the most abundant aldehyde. Terpenes and alcohols have been reported to have strong antibacterial effects, and (E) -2-hexenal has good inhibitory activity against bacteria and fungal pathogens (Wang et al., 2018; Feng, 2010). These ingredients may play an important role in rice dumpling having a longer shelf life. The results indicate that fresh *Indocalamus latifolius* leaves has good application prospects in the field of antiseptic materials and food packaging. Based on the exploration of volatile components of fresh *Indocalamus latifolius* leaves, the purpose of this article is to provides theoretical basis to develop and utilize the value of *Indocalamus latifolius* leaves.

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