DEVELOPMENT OF BIOACTIVE COMPONENTS FROM *CHAENOMELES SINENSIS* LEAVES

by

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Due to its good ornamental value, Chaenomeles sinensis has a long history of planting in China, and its fruit has health efficacy. The components from fruit and the functions of them have been fully excavated and developed, but there is little research on Chaenomeles sinensis leaves. In our study, the components of Chaenomeles sinensis leaves were extracted by ethanol and acetone. The extracts were identified by FT-IR and GC-MS in order to detect and excavate the bioactive components. The result shows that Chaenomeles sinensis leaves contain alcohols, ethers, aldehydes and other substances which were identified by FT-IR. The main volatile organic compounds (VOC) of Chaenomeles sinensis immature leaves are alcohols, ketones, alkenes and alkanes. Some substances can be simultaneously extracted by two solvents. Ethanol extracts contain high content of biogenic substances, and acetone solvents can effectively extract bioenergy substances.

Key words: *Chaenomeles sinensis leaves, bioactive component, biomedicine component, bioenergy substance*

Introduction

Chaenomeles sinensis, belonging to Rosaceae, is widely planted in China especially in Shandong, Jiangsu, Anhui, Zhejiang and other places [1]. In recent years, with people's increasing awareness of environmental consciousness, *Chaenomeles sinensis* is often planted to return farmland to forestry. Sothe planting area expands rapidly and the output increases. Not only can it produce huge economic benefits, but it is also ornamental [2]. At the same time, *Chaenomeles sinensis* is often used in urban greening. In actual production, due to its hard wood, *Chaenomeles Sinensis* is commonly used as a bed column.

The fruit of *Chaenomeles sinensis* is large, golden and rich in aroma [3], but it tastes sour, so it usually needs to be cooked or marinated with sugar to eat. Although it tastes bad, it has high medicinal value and nutritional value. *Chaenomeles sinensis* fruit was rich in vitamins [4-6] and dietary fiber [7], organic acids and bioactive phenolic acids, as well as a large amount of bioactive pentacyclic triterpene acids [8], such as oleanolic acid and ursolic acid [9]. In addition, flavonoids are one of the major phytochemicals in the fruit of *Chaenomeles sinensis*, and are reported to be effective against antioxidant [10-12]. The fruit processing products are used as chemical raw materials, cosmetic raw materials, beauty products and antioxidant essences, and have very broad application prospects.

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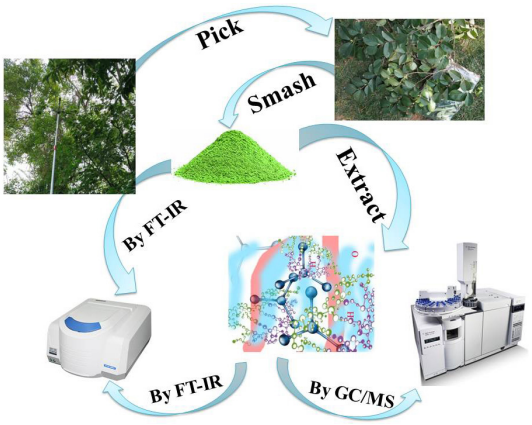

Figure 1. Preparation and analysis of *Chaenomeles sinensis* leaves extractives

At present, most studies focus on the development composition and function of *Chaenomeles sinensis* fruit. People have overlooked the leaves on *Chaenomeles sinensis*, which have very high yields each year. In this paper, *Chaenomeles sinensis* leaves are the key materials for research. The components of *Chaenomeles sinensis* leaves were detected by FI-RT and GC-MS, and the potential value of *Chaenomeles sinensis* leaves was systematically excavated, fig. 1.

Materials and methods

Materials and reagents

The leaves of *Chaenomeles sinensis* were collected from the *Chaenomeles sinensis* plantation of Henan Agricultural University. After fully dried at 40 °C by air blowing thermostatic oven, the samples were smashed into powder by using a FZ102 disintegrator suitable for plant (Tanjing Taisite Ins. Corp., China). Andthen, 200 mesh powders were sieved out.

Methods

Extraction by two solvents

Chaenomeles sinensis leaves were extracted by ethanol and acetone, respectively, with the ratio of solid-liquid = 1:30. After soaking for 8 hours at room temperature, the mixed samples were completely extracted by an automatic FOSS Soxhlet Extracted apparatus (Agilent, USA) at 80 °C, 56 °C for 3-5 hours, and then filtrated rapidly with filter paper immersed in ethanol, petroleum ether and acetone, respectively for 24 hours. The filtrated extraction was evaporated under vacuum at 45-50 °C, 0.01 MPa, and finally concentrated to 20 mL. The extracted residues were dried at 45-50 °C. All extracts and extracted residues were stored at 4 °C [13-16].

Analysis of group changes during extraction by FT-IR

The powders of *Chaenomeles sinensis* leaves, and their extracted residues were dried at 100 °C for 4 hours, and then put in the dry container with desiccant to prevent moisture absorption, so that not to affect the detection. A certain amount of potassium bromide were ground and sieved out with an AS200 Sieving Instrument (USA), placed in a dry pot, and then keep it in a muffle furnace (with SX-2.5-10 box-type control resistance furnace control box) at 150 °C for 5 hours, after that, it is removed under a heating lamp cover. 200 mg of potassium bromide was added to an agate mortar with a smooth surface, and 0.5-2 mg of the sample was mixed quickly and completely with potassium bromide in the mortar, and then placed in the tablet press for tabletting. As a liquid solution, the extracts were directly placed in the tablet press for tabletting. The pressed samples were tested in a Fourier transform infrared spectroscopy (SHIMADZU, IR Affinity-1) from 4000-400cm^{-1} [17-19].

Component analysis by GC-MS

The characteristic and relative content of components from extracts were analyzed by GC-MS.

GC condition: quartz capillary column was 30 mm × 0.25 mm × 0.25 μm, started at 50 °C, then followed by a rate of 8 °C/min up to 250 °C, finally reached 300 °C at a rate of 5 °C/min. The temperature of the inlet was 250 °C, column flow was 1.0 mL/min, split ratio was 20:1, and the carrier gas was high-pure helium [20, 21].

MS condition: ionization mode was EI, the electron energy was 70 Ev, the temperature of ion source was 230 °C, the temperature of quadrupole was 150 °C, and the starting scanning point was 30-600 [22].

Results and analysis

Chemical Group change characteristics of raw powders and extracts

Fourier transform infrared spectroscopy (FT-IR) is a mean of effectively identifying and analyzing the structure of substances. It has the advantages of fast, high sensitivity and simple operation. The original powder of *Chaenomeles sinensis* leaves and the extracts by two solvents of *Chaenomeles sinensis* leaves (acetone and ethanol) were determined by FT-IR to reveal the changes of chemical groups during extraction [23].

There are 10 main absorption peaks in FT-IR of *Chaenomeles sinensis* leaves powder, fig. 2(a). There is an absorption peak near 892 cm^{-1}, which represents an out-of-plane bending vibration of C-H, it can be inferred that there are olefins. The C-O stretching vibration mainly has absorption peaks of 1058 cm^{-1}, 1101 cm^{-1}, and 1260 cm^{-1}, which means that alcohols exist. There are absorption peaks near 1247 cm^{-1} and 1321 cm^{-1}, indicating C-O single stretching vibration, so it is possible to infer that there are aromatic ethers and phenols. At 1654 cm^{-1}, there is C=O stretching vibration, and it is speculated that there may be amides. There is a main absorption peak in 2933 cm^{-1}, here is C-H_2 antisymmetric stretching vibration, presumably alkanes. It can be speculated that *Chaenomeles sinensis* leaves powder mainly contains hydrocarbons, alcohols, phenols, and ethers [24].

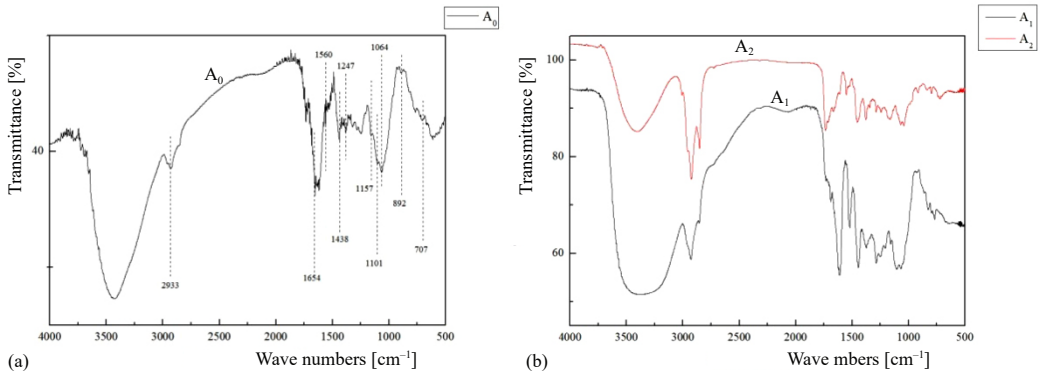

Figure 2. Infrared spectra of raw powders and its extracts of *Chaenomeles sinensis* leaves;
A_0*: raw powders,* **A_1** *ethanol,* **A_2***: acetone, extracted*

The FT-IR of ethanol extract from *Chaenomeles sinensis* leaves has 12 main absorption peaks, fig. 2(b). The C-O stretching vibration mainly has absorption peaks of 1105 cm^{-1}, 1159 cm^{-1}, and 1205 cm^{-1}, which means that alcohol is present. There are two absorption peaks at 1249 cm^{-1} and 1285 cm^{-1}, indicating that there is a C-O single-span stretching vibration, and it can be inferred that there are ethers and phenols. At 1524 cm^{-1}, there is C=C stretching vibration. The infrared absorption peak is C=O stretching vibration at 1688 cm^{-1}, and C=O

symmetric stretching at 2855 cm^{-1}, both of which can be presumed to be alkanes. From the aforementioned, it is presumed that there are substances such as alcohols, phenols, and ethers in the ethanol extract.

Acetone extract has 13 main infrared absorption peaks, fig. 2(b). There are two absorption peaks at 1040 cm^{-1} and 1163 cm^{-1}, indicating C-O stretching, which can be predicted by alcohols. The absorption peak near 1286 cm^{-1} is C-O stretching vibration, and it is speculated that there are phenols. At 1524 cm^{-1}, there is C=C stretching vibration. The absorption peaks near 1683 cm^{-1} and 1737 cm^{-1} indicate that C=O and aldehydes and esters may exist. There is an obvious absorption peak near 2853 cm^{-1}, indicating that CH_2 has symmetrical stretching vibration, which means it has alkanes. The strong absorption peak near 2925 cm^{-1} indicates the existence of CH_2 antisymmetric stretching, probably alkane. We can speculate that acetone extracts mainly contain alcohols, alkanes, aldehydes and ethers.

Extracts of Chaenomeles sinensis leaves contain a variety of volatility liquid bioactive components.

The two extracts contain rich VOC including carbohydrates, alcohols, organic acids and so on, fig. 3. An area percentage of more than 0.5% was considered critical and was chosen for analysis [25].

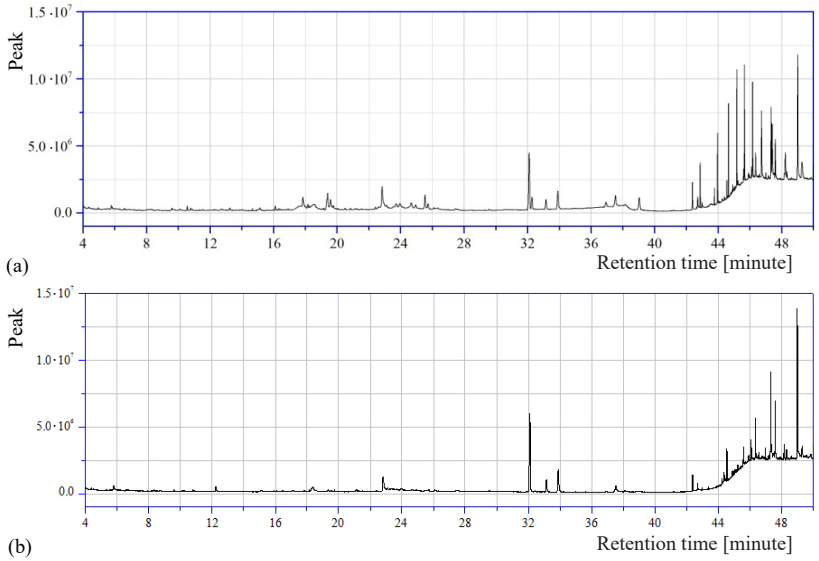

Figure 3. Ion chromatograms of ethanol (a) and acetone (b) extracts from *Chaenomeles sinensis* leaves by GC-MS

In the extract of ethanol, Phthalic acid, di(2,3-dimethylphenyl) ester (3.693%) is mainly used in polyvinyl chloride materials, which makes polyvinyl chloride change from hard plastic to elastic plastic, acting as plasticizer [26]. It is widely used in toys, food packaging materials, medical blood bags and rubber tubes, vinyl flooring and wallpapers, cleaners, lubricants, personal care products and other hundreds of products, but it has serious harm to human health [27]. Heptasiloxane, hexadecamethyl- (25.425%) is a biomass energy source that can be used for combustion [28].

In the extract of acetone, Maltose is used in biological media, polysulfide stabilizers, and analytical chemistry colorimetry to determine the brown standard [29]. Supraene is an open-chain triterpenoid compound. It was originally extracted from shark liver oil, which was then found in shark egg oil and other fish, and it is now found to be much more widely distribution than expected. Squalene is one of the intermediate of cholesterol biosynthesis, a precursor of all steroids [30]. It can be used to make nutritional medicine, internal treatment of high, low blood pressure, anemia and other diseases. It can be used in the synthesis of cholesterol biological intermediates, fungicides, pharmaceutical production of intermediates, aromatic agents. Caprolactam is an organic compound with the formula $(CH_2)_5C(O)NH$. Global demand for the compound is huge, reaching 5 million tons each year., and the vast majority is used to make Nylon 6 filament, fiber, and plastics. Phorone is used as a solvent for the production of synthetic resins, fibers, pharmaceutical intermediates, lubricant additives, and deodorants [31, 32].

The extracts of the two solvents contain some biomass energy substances. The highest content of ethanol extract was heptasiloxane, hexadecamethyl- (25.425%), and the highest content of acetone extract was neophytadiene (25.309%). Neophytadiene is inflammable and can form explosive mixtures by mixing with air. When Neophytadiene contacts heat, Mars, flame or oxidant, it is inflammable and explosive. It can be found that ethanol solvent is more effective than acetone solvent in the extraction of biomass energy substances.

As can be seen from tab. 1 that the volatile components are abundant, with a total of 7 different types of substances, and the extract content of different solvents is significantly different, tab. 2 and fig. 4. Ethanol extract of *Chaenomeles sinensis* leaves is mainly alkanes (accounting for 40.116% of the total), followed by alcohols (accounting for 14.709% of the total), and other contents are less. The relatively high content of heptasiloxane, hexadecamethyl- in alkanes is a good renewable biological energy source. The main volatile active constituents of *Chaenomeles sinensis* leaves acetone extract were alcohols and alkenes, which accounted for 29.428% and 26.663% of the total volatile components, respectively. By comparing and analyzing the relative content of acids and heterocycles in the volatile components of *Chaenomeles sinensis* leaves, it is more difficult to further develop. Comprehensive analysis, if you want to extract volatile alkanes active ingredients in *Chaenomeles sinensis* leaves, you should choose ethanol solvent. If you want to extract volatile alkenes and alcohols active ingredients in *Chaenomeles sinensis* leaves, you should choose acetone solvent.

Table 1. The Content of several biomass energy components

No	Compound name	Relative content [%]	
		Ethanol	Acetone
1	E-10-Dodecen-1-ol propionate	–	0.154
2	1,2,4-Tri-O-acetyl-3,5-di-O-methylribitol	1.563	–
3	2-Methoxy-6,10-dimethyl-dodeca-2E,6Z, 10Z-trienoic acid, 12-acetoxy-, methyl ester	2.75	–
4	Heptasiloxane, hexadecamethyl-	25.425	–
5	4-Methyl(trimethylene)silyloxyoctane	0.486	–
6	1-Chloromethyl-1-(2-propenyloxy)-1-silacyclohexane	0.187	–
7	Neophytadiene	10.211	25.309

Table 2. Component comparison of extracts from *Chaenomeles sinensis* leaves under different solvents

Solvent / Material / Relative content	*Chaenomeles sinensis* leaves Ethanol extractive	*Chaenomeles sinensis* leaves Acetone extractive
Esters	6.443	3.117
Acids	1.738	1.317
Alcohols	14.709	29.428
Ketones	1.339	15.541
Heterocycles	2.385	1.647
Alkenes	10.211	26.663
Alkanes	40.116	1.541

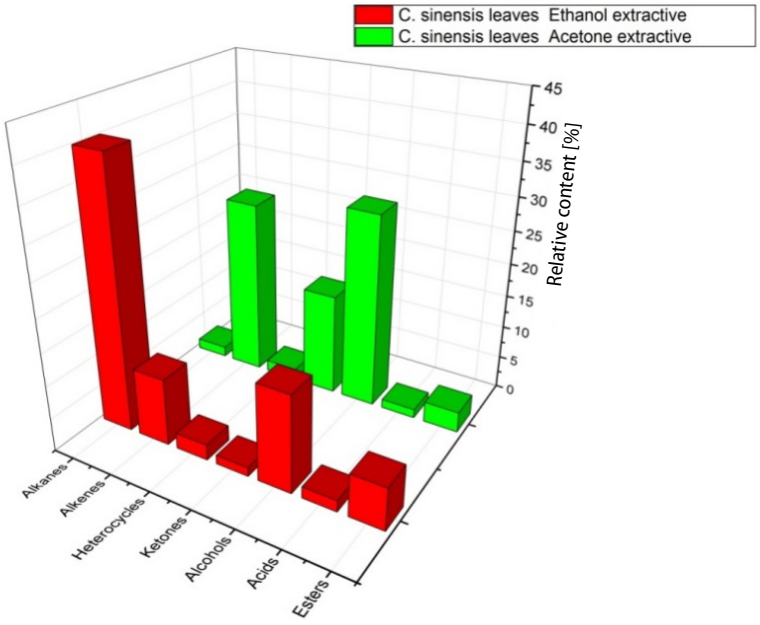

Figure 4. Different types of compounds of extracts from *Chaenomeles sinensis* leaves

Discussions and conclusion

According to GC-MS analysis, 108 and 71 components were identified from ethanol extract and acetone extract of *Chaenomeles sinensis* leaves, among which the main volatile active components could be identified as Esters, Acids, Alcohols, Ketones, Heterocycles, Alkanes and Alkenes. This is consistent with the results of FT-IR, and the results of FT-IR further confirm that the original samples of *Chaenomeles sinensis* leaves and extracting active ingredients are consistent. The chemical structure of *Chaenomeles sinensis* leaves is not significantly changed by organic solvent extraction.

Ethanol extract contains high content of biomass energy substances. The extracts of *Chaenomeles sinensis* leaves contain abundant bio-energy components, such as heptasiloxane, hexadecamethyl-, which has higher content in ethanol extracts, and the active components of

medical components, such as dl-. alpha. - Tocopherol, which are contained in both solvent extracts. Food ingredients and cosmetic raw materials also have some active ingredients, but the content is relatively low. Acetone solvents can effectively extract bioenergy substances. Some substances are high in content, such as octatriacontyl pentafluoropropionate, octasiloxane, 1, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl-, but the functions of these substances are not defined, which requires further exploration and study.

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