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Study on Active Ingredient of Ailanthus Leaves

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The separation and purification of active ingredient of *Ailanthus altissma* leaf and the analyze compositions were performed. The active ingredient of *Ailanthus altissma* leaf is separated by thin layer chromatography followed by GC-MS. The results showed that the active ingredients of the leaf could be isolated successfully by using the techniques of TLC with ethyl acetate:petroleum ether:methanol = 25:71:4 as the developing solvent. The GC-MS showed that the major ingredient of leaf is 9,12-diene-octadecane acid with abundant alkylate, such like decane, tetradecane, heptadecane, octodecane, heneicosane and some of esters like phthalic isobutyl alcohol ester, 8,11-diene-18 methyl ester and eighteen methyl esters, *etc*.

Keywords: *Ailanthus altissma*, TLC, GC-MC.

INTRODUCTION

Ailanthus altissima commonly known as tree of heaven, in Standard Chinese as chouchun is a deciduous tree in the Simaroubaceae family. It is native to both northeast and central China and Taiwan. *A. altissima* is a medium-sized tree that reaches heights between 17 and 27 metres with a diameter at breast height of about 1 metre. The leaves are large. They range in size from 30 to 90 cm in length and contain 10-41 leaflets organised in pairs. The leaflets are ovate-lanceolate with entire margins, somewhat asymmetric and occasionally not directly opposite to each other. Each leaflet is 5 to 18 cm long and 2.5 to 5 cm wide. They have a long tapering end while the bases have two to four teeth, each containing one or more glands at the tip. The leaflets' upper sides are dark green in colour with light green veins, while the undersides are more whitish green. The flowers are small and appear in large panicles up to 50 cm in length at the end of new shoots.

Recent research determined that the extract could apply in amebicide[1] and insecticide[2] as well. Other than those, a new test shows that the extract had prominence control effect on tobacco mosaic virus[3]. They found that the extract not only inhibits the spread of infection, but also controls the speeds of the virus to reproduce.

Lv[4], determined that components of the bark of *A. altissima* which was extracted by Soxhlet extraction and with diethyl ether as solvent, played a repellent effect on *Linpoxcelis paeta* (Pearman). A bioactivity experiment on tobacco pests further shows the status of the research[5]. In brief the methodology is as: diposed of samples 1, 2 and 3 day, respectively, the repellent rate accounted for 93.71, 87.75 and 76.14 %. Even the contact toxicity of extract is feeble to against tobacco beetle, there is a significant suffocating effect to the tobacco beetle. There is a high adjust mortality ratio, about 100 %, when the concentration of extract upper than 1.50×10^{-4} and processing under suffocation for 48 h.

In test of toxicology, Cao *et al.*[6] determined that the active ingredients in water, ethanol, diethyl ether and acetone extract of tree bark and leaf had evident toxic effect against the longicron pest[6]. In comparison, we found that the acetone extract had the best toxic effect and the diethyl ether could be the best solvents that extract active ingredients from plant and its ability of extracting active ingredients is superior to others.

EXPERIMENTAL

A. altissima leaves were collected from Beijing Union University College of chemical engineering, biological Baicao garden collection. Ethanol, chloroform, ethyl acetate, acetone, petroleum ether, methanol, formic acid and silica gel G were procured from silica gel G Beijing chemical reagent company.

Rotate evaporator RE-52 A, Dual wavelength UV lamp, (Shanghai biochemical instrument factory), Electric heating sleeve: model number ZDHW (Beijing zhongxingweiye Instrument Co. Ltd), chromatography column Φ2 cm × 70 cm, chromatography cylinder: size 150 × 100 × 200 mm; gas chromatography-mass spectrometry: GCMS-QP2010 Shimadzu, glass capillary (hard neutral glass): bore size 0.9-1.1 mm, wall thickness 0.10-0.15 mm, length 100 mm (Instrument factory

of West China University of Medical Sciences), thin layer plate: the self-made; U-1810 PC double beam UV-Vis spectrophotometer, Japan Shimadzu; constant temperature water bath oscillator: model number THZ-82; Shimadzu LC-10 ATVP high performance liquid chromatograph, Shimadzu Shimadzu-CLASS-VP.V6.12. SP4 workstation; electronic balance: BS210S, (Beijing Saiduolisi balance limited production); three ultraviolet analyzer (Shanghai Kang Huasheng instrument factory); sprayer (Zhejiang Huangyan City, sprayer Co. Ltd.); circulating water type multipurpose vacuum pump: SHB-95 (Henan Yuhua Instrument Co. ltd.)

VARIAN Prosta 210 Preparative chromatography workstation, C18 VARIAN Dyamax 250* 21.4 mm (L*ID).

Extraction of major components of the leaf: Clean off the dirt with water and stored it in a cool dry place. Once the leaf starts to dry, chopping it in patches. The leaf is extracted by ethanol through heating reflux about 2 h then purified by rotary evaporator

Separated by TLC: Using the silica gel G as the adsorbent mix with the water (1:3). When it turns out to be mushy, spread it on the layer about 2 mm thickness. Using the capillary absorbs the sample and drops it on the layer. Putting the ethyl acetate, methanol, petroleum ether together as the developing solvent and then pouring the developing solvent in chromatography cylinder. After about 20 min that the developing solvent was saturated, putting the complete layer in and waiting for 20 min approximately. Observe the location and the color of spot under the UV.

GC-MS analysis: Shimadzu Corporation GC-MS QP2010 gas chromatography mass spectrometry; gas chromatography conditions: column type: DB-5 ms 30 m, 0.25 mm; column temperature programmed conditions: initial temperature 80 °C, keep the time 2 min, 20 °C/dL to 250 °C, keep the time: 30 min; split ratio:10:1; inlet temperature: 250 °C. Mass spectrometric conditions: ion source temperature: 250 °C; electron energy: 0ev; scanning range: 20-650 *m/z*.

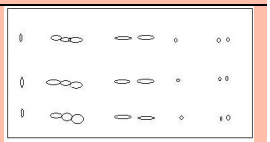
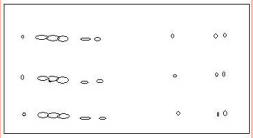
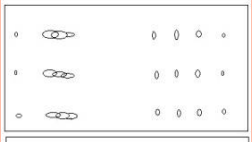
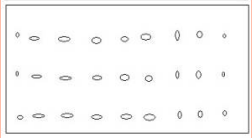
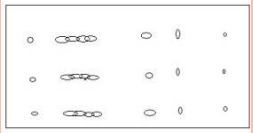
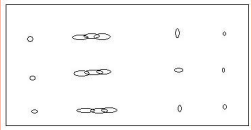
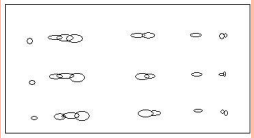
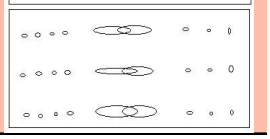
RESULTS AND DISCUSSION

Chromatographic separation of active ingredients: The separation of TLC is choosing appropriate developing solvent, which depending on the activity of adsorbent and the polarity of separated samples. Because of different action of various components in a developing solvent, in the layer, the larger polar solvent could moves compounds further, the small polar solvent that could drops off the elusive power and the R_f value of the larger polar solvent, in the medium polar solvent, two solvents which had vast polar difference were mixed uniformly. According to the theories of chromatography, the polarity of the developing solvent must close to the separated components in order to get a superior effect of separation.

The result of using ethyl acetate, petroleum ether, methanol as mobile phase are proved by mass of preliminary experiment results and the separating effect was evident by means of changing the proportion of them. The chromatography result of *A. altissima* leaf extracts in different solvent systems is shown in the Table-1.

This graph shows that the effective components of *A. altissima* leaf could be separated sufficiently by using ethyl acetate (6 mL), petroleum ether (17 mL) and methanol (1 mL) as the developing solvent. Sorted spots which appeared on the layer in ascending order from 1 to 8. When the developing solvent evaporated, scooping the silica gel which corresponding to every spot and then dissolved it in ethyl acetate. After filtration and precipitation, preserving the solution in penicillin bottle. Those above samples would be analyzed by GC-MS.

TABLE-1
ACTIVE INGREDIENT OF *A. altissima* LEAF ISOLATED BY TLC

Expand system (v/v/v)	Expand the case	Expand results
Ethyl acetate: petroleum ether: methanol = 6:16:1.5	Spots huddled together	
Ethyl acetate: petroleum ether = 8:16	Spots huddled together	
Ethyl acetate: petroleum ether: methanol = 5:16:1	Spots huddled together	
Ethyl acetate: petroleum ether: methanol = 6:17:1	Four spots clear separation	
Ethyl acetate: petroleum ether: methanol = 8:17:1.5	Spots huddled together	
Ethyl acetate: petroleum ether = 5:17	Spots huddled together	
Ethyl acetate: petroleum ether = 6:15	Spots huddled together	
Ethyl acetate: petroleum ether: methanol = 8:15:1	Unexpanded	
Ethyl acetate: petroleum ether: methanol = 5:15:1.5	Severe tailing	

Analysis of active ingredient of *A. altissima* leaf by GC-MS: We used GC-MS by standard material to identical all kinds of chromatograph peaks of compounds. Calculate the percentage composition of all kinds of compounds, according to peak area normalization method. According to the performance of the test, the DB-5 capillary has the advantage of both non-polar column and polar column and indicated a better separating effect. Direct sample injection method are adopted in accordance with the condition of analysis as above and then established a chromatographic fingerprint of GC-MS (Fig. 1).

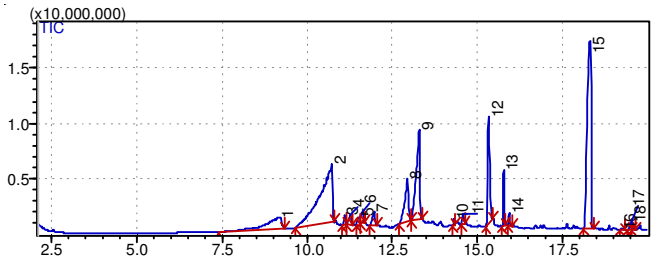


Fig. 1. Ingredient of *A. altissima* leaf of total ion current (TIC) chromatogram

Through analyzing by GC-MS technology, 45 chromatographic peaks were separated and 18 compounds were identified. The main constituents are long-chain higher fatty acids and their esters and a mass of alkylates. The data was processed by a chemical workstation and the identified components are shown in Table-2.

TABLE-2
ANALYTICAL RESULTS OF SAMPLE BY GC-MS

No.	Chemical composition	m.f.	m.w.
1	Decane	$C_{10}H_{22}$	142
2	Tetradecane	$C_{14}H_{30}$	198
3	2,6-*bis*(1,1-Dimethyl-ethyl)-4-methyl-phenol	$C_{15}H_{24}O$	220
4	Nonadecane	$C_{19}H_{40}$	268
5	Octadecane	$C_{18}H_{38}$	254
6	Heneicosanoic	$C_{21}H_{44}$	296
7	Phthalic acid-2-Methyl alcohol-two ester	$C_{16}H_{22}O_4$	278
8	Sixteen alkyl acid	$C_{16}H_{32}O_2$	256
9	9,12-Diene-eighteen alkanoic acid	$C_{18}H_{32}O_2$	280
10	Hexacosane	$C_{26}H_{54}$	366
11	Hexatriacontane	$C_{36}H_{74}$	506
12	Tetracosane	$C_{24}H_{50}$	338
13	Tetratriacontane	$C_{34}H_{70}$	478
14	Heptadecanoyl	$C_{17}H_{36}$	240
15	Phthalic acid isobutyl alcohol two ester	$C_{16}H_{22}O_4$	278
16	Seventeen acid methyl ester	$C_{17}H_{34}O_2$	270
17	8,11-Diene-eighteen acid methyl ester	$C_{19}H_{34}O_2$	294
18	Methyl stearate	$C_{19}H_{38}O_2$	294

Making analysis and study on components of *A. altissima* leaves and we realized that 9,12-diene-eighteen alkanoic acid could treat arteriosclerosis and high blood lipid. The allelopathy of the phthalic acid isobutyl alcohol two ester on verticillium wilt of eggplant and seedling growth. The antibacterial, anti-viral effect of 3-chloro-octane is superior to berberine, mequindox and ofloxacin obviously and same as the florfenicol, otherwise, has antitumor effects[3]. Sixteen alkyl acid could inhibited the effects on inflammation and pain[7].

Isolation of components by preparative chromatography: On the base of research in TLC, the Ailanthus leaves extracts could be concentrated the extracts to 10 mL by the rotary evaporation in 75 °C. Using Varian prosta 210 preparative chromatograph with C_{18} column and mobile phase of ethyl acetate, petroleum ether, methanol (25:71:4), flow rate of 2 mL/min, detection wavelength of 220 nm and 30 °C as column temperature. Collected the separated chemical components by means of interceptor technique, preparative chromatography (Fig. 2) There are seven components were separated and purified.

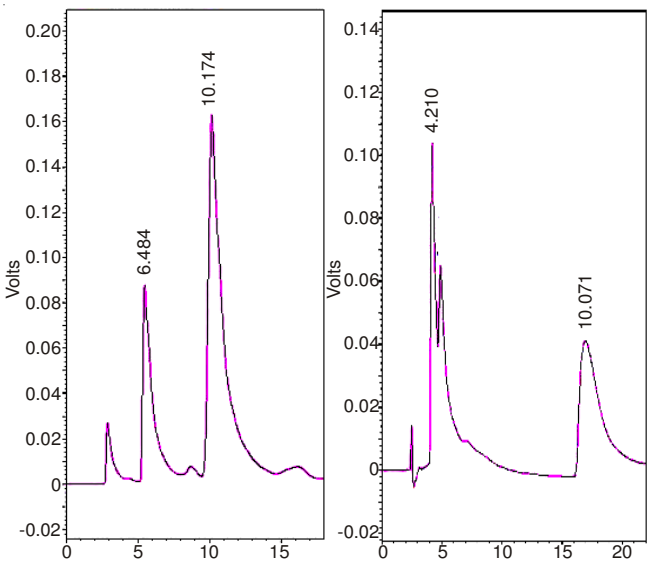


Fig. 2. Ingredient of *A. altissima* leaf isolated by preparative chromatography

Conclusion

According to the analysis by GC-MS, it is observed that the major active ingredients of *A. altissima* leaf are long-chain alkane, long chain fatty acids and their esters. The content of 9,12-diene-octadecane acid is highest.

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