Journal of Ethnopharmacology 141 (2012) 187–192

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Identification and quantification of the major volatile constituents in antidepressant active fraction of xiaoyaosan by gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 1 September 2011
Received in revised form 13 January 2012
Accepted 2 February 2012
Available online 17 February 2012

Keywords:
Active fraction
Depression
Gas chromatography–mass spectrometry
XYS

ABSTRACT

Ethnopharmacological relevance: Xiaoyaosan (XYS), a well-known formula for relieving depression, was originated from the book of "Taiping Huimin Heji Jufang" in Song Dynasty (960–1127 AD), composed of *Radix Bupleuri*, *Radix Angelicae Sinensis*, *Radix Paeoniae Alba*, *Rhizoma Atractylodis Macrocephalae*, *Poria*, *Herba Menthae*, *Rhizoma Zingiberis Recens* and *Radix Glycyrrhizae* with dose proportion of 6:6:6:6:6:3:2:2. It is commonly used for the treatment of depression-related syndromes in China. In the formula, *Radix Bupleuri* usually serves as the principal drug, *Radix Angelicae Sinensis* and *Radix Paeoniae Alba* serve as the ministerial drugs, *Rhizoma Atractylodis Macrocephalae*, *Poria*, *Herba Menthae* and *Rhizoma Zingiberis Recens* serve as adjunctive drugs, *Radix Glycyrrhizae* serves as messenger drug, they coordinate with each other and enhance the effect of the formula. In our previous experiments, the antidepressant effect of XYS was revealed. However, the antidepressant part (or component) of this prescription was still obscure.

Materials and methods: An experimental despair animal model: the mice tail suspension test (TST) was used to evaluate the antidepressant activity of XYS and its fractions. GC–MS method was developed to identify the volatile components and determine 4 major volatile components in active fraction.

Results: In the TST test, the effect of a low polar fraction (XY-EA) was superior to other fractions of XYS. 13 volatile compounds in the XY-EA were identified on the basis of standards, isolation and structural determination in our laboratory, NIST 05 database and literature data. The content of 4 major volatile compounds in XY-EA which is 6.703%.

Conclusions: The petroleum ether fraction (XY-EA) appears to be the active fraction of XYS. 4 major components *Z*-ligustilide, palmitic acid, atractylenolide I, and atractylenolide II may be the antidepressant active compounds.

1. Introduction

Depression is the complex psychiatric disorder characterized by anhedonia and feelings of sadness. It was estimated by National Institute of Mental Health (NIMH) that more than 12 million women and 6 million men in the USA are affected by depressive illnesses in any given year (Rudy, 2004). Furthermore, the WHO revealed that depression is the fourth leading cause of disability worldwide, exceeded by lower respiratory infections, perinatal conditions and HIV/AIDS (WHO, 2001).

XYS, a valuable traditional Chinese medicine, is composed of the following eight raw materials: *Radix Bupleuri* (*Bupleurum chinense DC.*), *Radix Angelicae Sinensis* (*Angelica sinensis (Oliv.) Diel*), *Radix Paeoniae Alba* (*Paeonia lactiflora Pall.*), *Rhizoma Atractylodis Macrocephalae* (*Atractylodes macrocephala Koidz.*), *Poria* (*Poria cocos (Schw.) Wolf*), *Herba Menthae* (*Mentha haplocalyx Briq.*), *Rhizoma Zingiberis Recens* (*Zingiber officinale Rosc.*) and *Radix Glycyrrhizae* (*Glycyrrhiza uralensis Fisch.*). Even though it has been reported that XYS showed significant antidepressant effect (Xiong et al., 2007), the efficacious material basis of XYS in producing significant antidepressant-like effect has not been reported.

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doi:10.1016/j.jep.2012.02.018

It is difficult to mimic depression in the laboratory, because depression-related syndromes are associated with multifaceted symptoms which manifest themselves at the psychological, behavioral and physiological levels. The TST was the most widely accepted model for assessing antidepressant-like activity in mice. The immobility taken on in this test is understood to reflect a state of 'behavioral despair and variants' (Willner, 1997). Antidepressants can also be distinguished from stimulants because stimulants cause marked motor simulation, in contrast to antidepressants, which do not (Borsini and Meli, 1998).

In this study, the antidepressant effect of six different fractions was evaluated on a despair animal model: the mice tail suspension test (TST), accompanied with the analysis by GC–MS to identify the volatile components and determine 4 major volatile components in active fraction.

2. Materials and methods

2.1. Plant material

Radix Bupleuri (Hebei; 2009; 0908001), *Radix Angelicae Sinensis* (Gansu; 2008; 0908002), *Radix Paeoniae Alba* (Anhui; 2009; 0908003), *Rhizoma Atractylodis Macrocephalae* (Zhejiang; 2009; 0908004), *Poria* (Anhui; 2008; 0908005), *Herba Menthae* (Hebei; 2009; 0908006), *Rhizoma Zingiberis Recens* (Sichuan; 2009; 0908007), *Radix Glycyrrhizae* (Gansu; 2009; 0908008) were purchased from Shanxi Huayang Pharmaceutical Company and authenticated by Prof. Xue-Mei Qin, Shanxi University, before preparation.

The venlafaxine (KangHong pharmaceutical, No. 071104) were purchased from HuangHe drugstore (Taiyuan, China).

Z-ligustilide, palmitic acid, atractylenolide I and atractylenolide II were purchased from Shanghai Shunbo Co. Ltd. (Shanghai, China).

2.2. Preparation of the extracts

As shown in Scheme 1 the traditional Chinese formula xiaoyaosan was extracted with anhydrous ethanol, and the extract was partitioned with extraction and D101 macroporous resin column to afford XY-EA, XY-EB_1, XY-EB_2 and XY-EB_3 fractions. The w/w extractions yields were 0.52%, 2.0%, 0.39%, 0.5% respectively. The residue of the crude drug was extracted with water, and the extract was partitioned with D101 macroporous resin column to afford XY-C_1 and XY-C_2. The w/w extractions yields were 0.9% and 2.11%.

A second sample of the crude drug was following: eight mixed plant materials weighted about 150 g were extracted with boiling water for 2 h and this procedure was repeated twice, the combined decoction was dried in vacuo and grinded into powder served as the positive control (XYS). The w/w extractions yield was 13%.

2.3. Animals

Male ICR mice (20–22 g) were used in the TST. All the animals used in this study were cared for and treated humanely according to the 'Principles of Laboratory Animal Care' (NIH Publication No. 85-23, revised in 1985). The animals were housed for 1 week under controlled conditions before the experiments took place. These conditions were as follows: light (12 h light/dark cycle, lights on at 7:00 am), temperature ($25 \pm 1\,^{\circ}C$), free access to food and water. The animals were randomly assigned to 9 equal groups for the experiments: vehicle solvent control group (VEH), XY-EA group, XY-EB_3 group, XY-EB_2 group, XY-EB_1 group, XY-C_1 group, XY-C_2 group, XYS group and a group for venlafaxine. Each experimental group consisted of ten mice. All the animals were purchased from the Experimental Animal Center of Military Medical Sciences Academy (No. SCXK 2007-004).

2.4. Drug administration

All drugs were dispersed in 1% CMC-Na added Tween 80 (0.5%, w/v). Vehicle solvent (0.5%, w/v Tween 80 dissolved in 1% CMC-Na) served as negative control. VLF (administered at 50 mg/kg) and XYS served as positive control groups in TST. The solutions of the tested samples were administered to the mice via gastric intubation at a dosage of 0.4 mL/20 g (body weight) once a day at 9:00 am for 14 days. The amounts of XYS and each fraction administered to the groups were 5980, 240, 720, 180, 240, 400 and 920 mg/kg, which were calculated according to effective dose of XYS obtained from previous experiment (Dai et al., 2010) and yield of extracts. The locomotor activity was measured twice in the mice before the first administration and 50 min after the final administration respectively. The behavioral tests on the mice were started 60 min after the final administration.

2.5. Antidepressant activity evaluation

2.5.1. Measuring locomotor activity

In order to detect any link between immobility in the TST and changes in motor activity, the activity level of animals was analyzed in the mice locomotor activity recorder apparatus (ZZ-6, Chengdu Taimeng Technology Co., Ltd.). The mice were placed in the apparatus for 8 min. During this period, measurements were taken only in the final 5 min. The apparatus was cleaned after each test session to prevent each mouse from being influenced by the odors present in the urine and faces of the previous mouse.

2.5.2. Tail suspension test

The mouse was hung by the tail (clipped 2 cm from the end) for 6 min in a box of dimensions 50 cm × 25 cm × 50 cm, its head 15 cm above the bottom of the box. Data was recorded only in the final 4 min of the test (Steru et al., 1985). 'Immobility' was scored as a failure to make any struggling movements. During the test session, the immobility time was recorded using a video camera. Two observers who had no knowledge of the type of treatment each animal had received evaluated the tapes.

2.5.3. Statistical analysis

Data were reported as the means ± S.E.M. Overall differences according to the treatment were confirmed using the one-way analysis of variance (ANOVA). Analysis of variance and least significant difference tests (SPSS for Windows, Version rel. 11.5, SPSS Inc., Chicago, IL) were conducted to identify differences among means. Statistical significance was declared at $p < 0.05$.

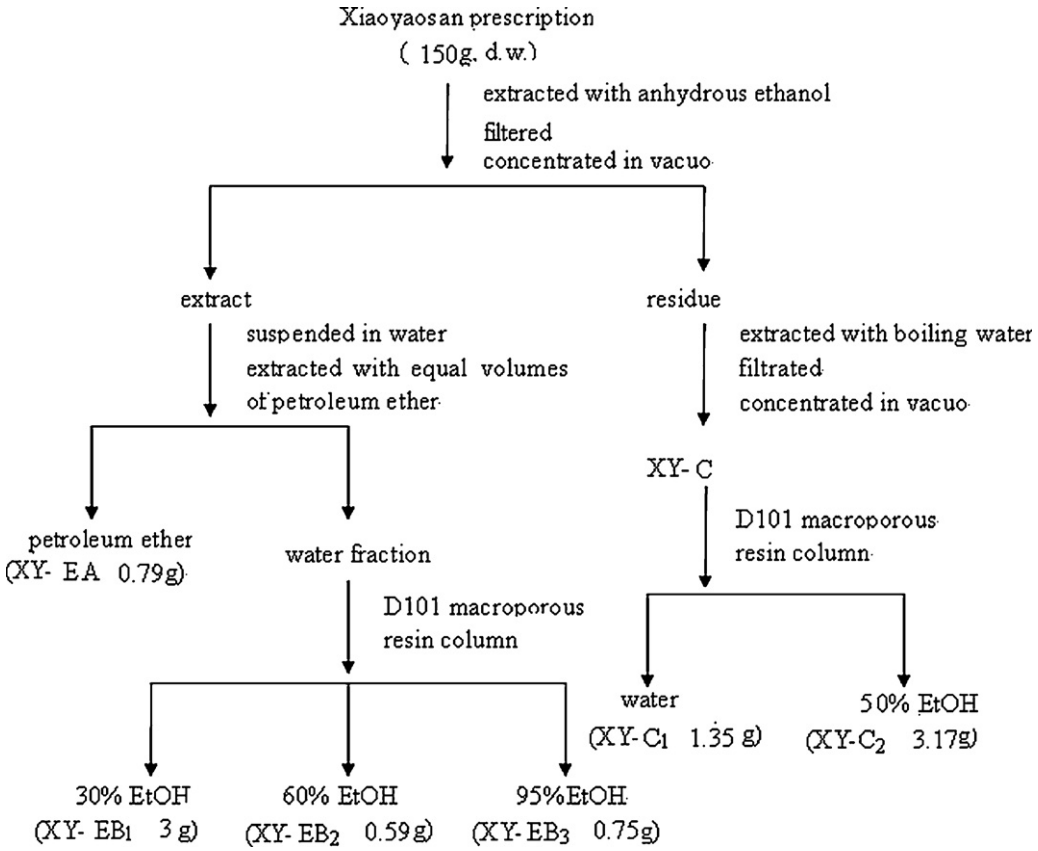
2.6. Preparation of solutions in GC–MS

2.6.1. Preparation of standard solutions

Stock solutions of the 4 standard substances for the determination were prepared in methanol at the concentration (mg/ml) of: *Z*-ligustilide (0.36), palmitic acid (0.20), atractylenolide I (0.03) and atractylenolide II (0.02). The prepared mixed standard stock solution containing 4 analytes was diluted to a series of appropriate concentrations for the construction of calibration curves. Seven different concentrations of the mixed standard solution were injected in triplicate. All the solutions were stored in the refrigerator at 4 °C and brought to room temperature before use.

2.6.2. Preparation of sample solutions

XY-EA samples for determination were gained by extracting for six times parallelly as described in Section 2.3. Each sample was



Scheme 1. The process of partition of xiaoyaosan.

injected in triplicate which was dissolved in petroleum ether and acetone and was filtered through a 0.45 μm membrane.

2.7. GC/MS analysis

GC/MS analysis was performed using an Agilent 7890 gas chromatograph, 5975 mass spectrum detector (MSD) (Agilent Technologies, Palo Alto, CA). Chromatography was performed on a HP-5 MS capillary column (30m × 250 μm *i.d.*, 0.25 μm film thickness; 5% diphenyl cross-linked 95% dimethylpolysiloxane; Agilent J&W Scientific, Folsom, CA). Split injection (1 μl) was conducted with a split ratio of 5:1. To acquire a well separation, the column temperature was programmed to rise from an initial temperature of 50 °C (2 min) to 120 °C at 15 °C/min, then to 150 °C at 3 °C/min, then to 200 °C at 7 °C/min, then to 230 °C at 3 °C/min, then to 280 °C at 15 °C/min and held at 280 °C (1 min). The injection, interface, and source temperatures were set at 200 °C, 280 °C and 200 °C respectively. Helium was used as the carrier gas with a flow rate of 1.0 ml/min. After a solvent delay of 5 min, MS detection was implemented with electron ionization mode (electron energy of 70 eV) and full scan mode (*m*/*z* 50–650). Compounds in the XY-EA were identified on the basis of standards, isolation and structural determination in our laboratory, NIST 05 database and literature data (Lao et al., 2004; Qin et al., 2007; Yang et al., 2005).

3. Results and discussion

3.1. Analysis of behavior test

The result has demonstrated that locomotion did not differ statistically among all groups.

The behavioral effects produced in the TST test owing to drug administration are presented in Fig. 1. A significant reduction in immobility time was observed in the mice treated with VLF, XYS, XY-EB$_3$, XY-EA versus VEH. The other groups showed no significant reduction. The results showed that XY-EA can significantly reduce the immobility time in the TST, indicating significant antidepressant effects in the animal model test. XY-EA which was superior to other fractions of XYS in the TST test was low polar fraction. It has been reported that Yueju-Wan and its petroleum ether fraction exert antidepressant effects in experimental

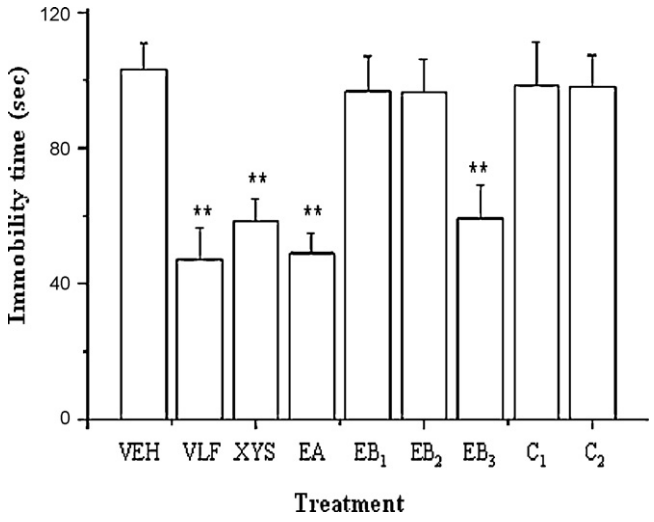


Fig. 1. Effects of different fractions of XYS in the TST. All the test substances were orally administered 60 min before the test. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle-treated group (VEH).

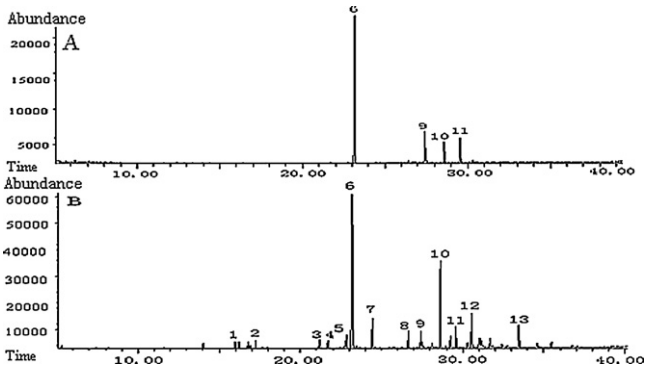


Fig. 2. GC–MS total ion chromatograms of (A) mixed standards (6) *Z*-ligustilide, (9) palmitic acid, (10) atractylenolide I, (11) atractylenolide II; (B) XY-EA.

animal models (Wei et al., 2008), and the total 90% ethanol extract of the Banxia Houpu decoction possess an antidepressant activity and the active constituents were lipophic parts (Luo et al., 2000). Banxia Houpu Decoction and Yueju-Wan (YJ), traditional Chinese medicinal formulas, are both commonly used for the treatment of depression-related syndromes in China. Banxia Houpu Decoction consisting of *Pinellia ternata*, *Poria cocos*, *Magnolia officinalis*, *Perilla frutescens* and *Zingiber officinale*. YJ is comprised of five indigenous medicines: *Cyperus rotundus* L., *Ligusticum chuanxiong* Hort, *Gardenia jasminoides* Ellis, *Atractylodes lancea* (Thunb.) DC. and *Prunus armeniaca* L. Our result also suggested the low polar molecule play an important role in antidepressant formula. Obviously, the XY-EA consists mainly of a mixture of essential oils and other lipophilic constituents, so GC–MS method was developed for analysis of the volatile compounds of it.

3.2. Identification of chemical components in XY-EA

The total ion chromatogram (TIC) of XY-EA is shown in Fig. 2. The sample was well separated in 40 min. 13 compounds identified in XY-EA are listed in Table 1.

Using GC–MS analysis, we found that liguistilide (from *Radix Angelicae Sinensis* and *Radix Bupleuri*), palmitic acid (from *Radix Bupleuri* and *Rhizoma Atractylodis Macrocephalae*), atractylenolide I and atractylenolide II (from *Rhizoma Atractylodis Macrocephalae*) were the main volatile components of XY-EA, while *Angelicae Sinensis*, *Bupleurum Chinense DC* and *Rhizoma Atractylodis Macrocephalae* are the main ingredients of the XYS formula. It has been reported that the main components of effective fraction of Yueju-Wan also included liguistilide (Luo et al., 2000), and liguistilide is primary component of *Angelicae Sinensis* essential oils (Zhou et al., 2007),

Table 2
Mass data of four components investigated in XY-EA.

Peak no	Compound	Mass data[a]
1	*Z*-Ligustilide	190 (M^+,74), 161 (100), 148 (90), 105 (95), 77 (50), 55 (40)
2	Palmitic acid	256 (M^+,40), 213 (32), 185 (20), 129 (60), 73 (100), 60 (80), 43 (80)
3	Atractylenolide I	230 (M^+,90), 215 (100), 201 (70), 91 (62), 77 (45), 39 (42)
4	Atractylenolide II	232 (M^+,100), 217 (60), 199 (50), 91 (69), 79 (48), 39 (15)

[a] (m/z) Relative intensity shown in parentheses, and the ion of relative intensity 100 was used for the quantification.

and could directly suppress the activity of CRF systems, or indirectly affect the GABAA receptor systems (both of the two effects may be related to the mechanism of depression) (Matsumoto et al., 1998). In addition, plamitic acid, one component of *Bupleurum Chinense DC* (Li et al., 2005) and *Rhizoma Atractylodis Macrocephalae* essential oils, has a peculiar position with respect to the biochemical characterization of Major Depression (Cocchi and Tonello, 2007). All of the above information suggests that the low polar molecule may play an important role in antidepressant activity of XYS. Therefore, these components were necessarily quantified for discovering a novel potential antidepressant and controlling the quality of it in future.

3.3. Quantitative analysis

The selected ion monitoring (SIM) method was used for the quantification of 4 components. A fragment ion m/z 161 was used for *Z*-ligustilide, m/z 73 for palmitic acid, m/z 215 for atractylenolide I and m/z 232 for atractylenolide II (Table 2). Their structures are presented in Fig. 3.

The calibration curves, which were obtained from the selected ions peak area, for *Z*-ligustilide, palmitic acid, aractylenolide I and atractylenolide II were linear over the range 3.56–177.8, 0.20–10.1, 0.29–14.5 and 0.25–12.5 ng respectively. The coefficients of correlation (R^2) were between 0.9902 and 0.9998. The results were shown in Table 3.

The injection precision for *Z*-ligustilide, palmitic acid, atractylenolide I and atractylenolide II was determined by injecting successively standard for six times. The relative standard deviation (R.S.D.) of *Z*-ligustilide, palmitic acid, atractylenolide I and atractylenolide II was 2.30, 2.76, 1.37 and 1.29%, respectively.

The stability of *Z*-ligustilide, palmitic acid, atractylenolide I and atractylenolide was also determined by injecting freshly prepared standard solution for three times at 0, 1, 2, 4, 8, 12 h, respectively.

Table 1
Compounds identified in XY-EA.

No.	Retention time	Compounds	Mass fragment
1	16.77	Germacrene D[c]	204, 161, 120, 119, 105, 91
2	17.24	Caryophyllene[c]	204, 161, 133, 119, 105, 93
3	21.19	γ-Elemene[c]	204, 133, 121,105, 77
4	21.71	*E*-Butylidenephthalide[c]	188, 146, 131,103, 91
5	22.85	Senkyunolide A[c]	192, 133, 107, 91, 85
6	23.19	*Z*-Ligustilide[a]	190, 161, 148, 105
7	24.45	*E*-Ligustilide[a]	190, 161, 148, 105
8	26.66	11-isoproylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one[c]	202, 187, 173, 159, 145, 131
9	27.52	Palmitic acid[a]	256, 213, 185, 129
10	28.63	Atractylenolide I[a]	230, 215, 201, 91
11	29.58	Atractylenolide II[a]	232, 217, 199, 91
12	30.55	(2*Z*,8*E*,10*E*)-Pentadecatriene-4,6-diyn-1-ol[b]	214, 185, 128, 115, 91
13	33.46	2,8,10-Pentadecatrien-4,6-diyn-1-ol, acetate[b]	256, 213, 171, 128, 115

[a] Identified by comparing with standards.
[b] Identified on the basis of separation, purification and structural ascertainment.
[c] Identified on the basis of NIST 05 database and literature data.

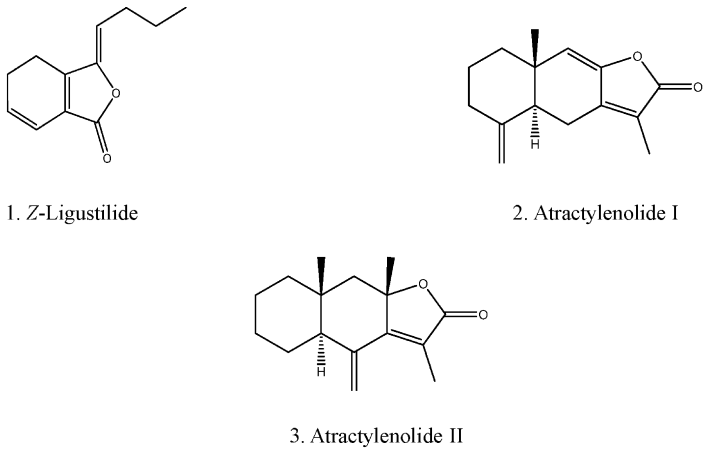


Fig. 3. Structures of investigated compounds in XY-EA.

Table 3
Linear regression data and recovery of investigated components in XY-EA.

Analytes	SIM	Linear regression data			LOD (ng)	Recovery (%, $n=3$)	
		Regressive equation	Linear range (ng)	r^2			R.S.D.
Z-Ligustilide	161	$y = 6E+06x - 8211.1$	3.56–177.80	0.9902	0.757	99.51	0.63
Palmitic acid	73	$y = 2E+07 - 15600$	0.20–10.1	0.9998	0.236	98.36	0.24
Atractylenolide I	215	$y = 4E+06 - 281.1$	0.29–14.50	0.9909	0.062	99.82	1.53
Atractylenolide II	232	$y = 5E+06 - 334.1$	0.25–12.50	0.9970	0.079	99.32	0.49

Table 4
The contents (mg/g) of four investigated compounds in XY-EA.

Compounds	Contents in XY-EA (mg/g)	RSD (%) ($n=6$) XY-EA
Z-Ligustilide	40.25	0.94
Palmitic acid	7.20	2.89
Atractylenolide I	13.36	0.85
Atractylenolide II	6.22	2.11

The R.S.D. of *Z*-ligustilide, palmitic acid, atractylenolide I and atractylenolide II was 2.13, 1.96, 1.73 and 1.82%, respectively.

The recovery was preformed by adding known amount of individual standards into an accurately weighed sample. The mixture was analyzed using the method mentioned above. The results were shown in Table 3.

3.4. Determination of Z-ligustilide, palmitic acid, atractylenolide and atractylenolide II in XY-EA samples

The samples were analyzed by the developed GC–MS method under selected conditions. The calibration curves were used for a quantitative analysis of the four compounds. The results were listed in Table 4.

It showed that the content of four investigated compounds in XY-EA which is the active antidepressant fraction is 6.703% so they may be the antidepressant active compounds.

4. Conclusions

In this study, the traditional Chinese medicinal formula Xiaoyao-San was separated into six different polar fractions. XY-EA exerts antidepressant effects in experimental animal model and it appears to be the fraction that contain the active constituents of xiaoyaosan. XY-EA is low polar fraction which consists mainly of lipophilic constituents. So GC–MS method was developed for analysis of the volatile compounds of it. The developed GC–MS method is simple and accurate for identification and determination of major volatile compounds in active fraction which is helpful for further research on antidepressant material basis of XYS. Of course, only volatile compounds in XY-EA can be detected by GC–MS, others cannot.

Therefore, other methods will be developed in future for comprehensive analysis of it.

This work has demonstrated that XY-EA (lipophilic components) is the active fraction of XYS, which may provide scientific evidence supporting XY-EA fraction to become a novel potential antidepressant. And the work also suggests that TCM could be served as the important sources to discover new anti-depressant drugs.

Acknowledgements

This study was financially supported by National Natural Science Foundation of China (No. 30901960), Programs of International Science and Technology Cooperation of China (No. 2008DFA30430), Programs of Science and Technology of Shanxi Province (No. 20100312031).

References

Borsini, F., Meli, A., 1998. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology 94, 147–160.
Cocchi, M., Tonello, L., 2007. Platelets, fatty acids, depression and cardiovascular ischemic pathology. Progress in Nutrition 9, 94–104.
Dai, Y.T., Li, Z.Y., Xue, L.M., Dou, C.Y., Zhou, Y.Z., Zhang, L.Z., Qin, X.M., 2010. Metabolomics study on the anti-depression effect of xiaoyaosan on rat model of chronic unpredictable mild stress. Journal of Ethnopharmacology 128, 482–489.
Lao, S.C., Li, S.P., Kan, Kelvin, K.W., Li, P., Wan, J.B., Wang, Y.T., Dong, Tina, T.X., Tsim, Karl, W.K., 2004. Identification and quantification of 13 components in *Angelica sinensis* (Danggui) by gas chromatography–mass spectrometry coupled with pressurized liquid extraction. Analytica Chimica Acta 526, 131–137.
Li, X.Q., Song, A.H., Li, W., Chen, X.H., Bi, K.S., 2005. Analysis of the fatty acid from *Bupleurum Chinense DC* in China by GC–MS and GC-FID. Chemical and Pharmaceutical Bulletin 53, 1613–1617.
Luo, L., Nong, W.J., Kong, L.D., Jiang, Q.G., Tan, R.X., 2000. Antidepressant effects of Banxia Houpu decoction, a traditional Chinese medicinal empirical formula. Journal of Ethnopharmacology 73, 277–281.
Matsumoto, K., Kohno, S., Ojima, K., Tezuka, Y., Kadota, S., Watanabe, H., 1998. Effects of methylenechloride-soluble fration of Japanese *angelica* root extract, ligustilide and butylidenephthalide, on pentobarbital sleep in grouphoused and socially isolated mice. Life Sciences 62, 2073–2082.

Qin, N.Y., Yang, F.Q., Wang, Y.T., Li, S.P., 2007. Quantitative determination of eight components in rhizome (*Jianghuang*) and tuberous root (*Yujin*) of *Curcuma longa* using pressurized liquid extraction and gas chromatography–mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 43, 486–492.

Rudy, M.B., 2004. The darkness of depression. Chemical and Engineering News (C & EN) 82, 5.

Steru, L., Chermat, R., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

Wei, X.H., Cheng, X.M., Shen, J.S., Wang, Z.T., 2008. Antidepressant effect of Yueju-Wan ethanol extract and its fractions in mice models of despair. Journal of Ethnopharmacology 117, 339–344.

Willner, P., 1997. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology 134, 319–329.

World Health Organization. The World health report 2001: Mental health: new understanding, new hope. Geneva.

Xiong, J.Y., Zang, N., Zhang, C.Y., Yang, J., Liu, X.S., 2007. Studies on the anti-depression effect of Xiaoyao Powder in mice. Pharmacology and Clinics of Chinese Materia Medica 23, 3–5.

Yang, F.Q., Li, S.P., Chen, Y., Lao, S.C., Wang, Y.T., Dong, Tina, T.X., Tsim, Karl, W.K., 2005. Identification and quantitation of eleven sesquiterpenes in three species of *Curcuma rhizomes* by pressurized liquid extraction and gas chromatography–mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 39, 552–558.

Zhou, Y.H., Xie, H., Wang, T.W., Bai, H.Q., 2007. Extraction of volatile oil from *Angelica* and its chemical components analysis. Technology and Development of Chemical Industry 36, 33–35.

Glossary

NIMH: National Institute of Mental Health
TST: the mice tail suspension test
VLF: venlafaxine
VEH: vehicle solvent control group
ANOVA: analysis of variance
GC–MS: gas chromatography–mass spectrometry
MSD: mass spectrum detector
TIC: the total ion chromatogram
SIM: the selected ion monitoring
TCM: Traditional Chinese Medicine