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# GC-MS Analysis of Chemical Components of *Taxus Chinensis Var.* Mairei Seeds

**Review Article**

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**Abstract**

The extracts of petroleum ether, methanol, ethyl acetate and ether from *Taxus chinensis var. mairei* seeds were analyzed by GC-MS, and relative contents were determined using a normalized method. 56 peaks were obtained from extracts of spermoderm and 79 peaks from endosperm of *T. chinensis* var. *mairei*. Among these peaks, 37 chemical constituents were gained, and 24 compounds of which were identified (64.86%). There were 35 chemical constituents with content more than 1%, accounting for 94.59% of the total of all chemical constituents. 32 peaks were gained from petroleum ether extract, and 7 peaks were identified (21.88%) with content more than 1%. 36 peaks were gained from methanol extracts, and 14 peaks were identified (38.89%), 13 of which with content more than 1%, accounting for 92.86% of the total of all chemical constituents. 34 peaks were gained from ethyl acetate extracts, while 10 peaks were identified (29.41%) with content more than 1%. 33 peaks were gained from aether extracts, and 11 peaks were identified (33.33%). *T. chinensis* var. *mairei* seeds have various bio-active ingredients and a higher value for development and utilization as a medicinal plant.

**Keywords:** *Taxus chinensis var. mairei*; seed; chemical components; GC-MS; composition analysis

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## Introduction

*Taxuschinensis var.*mairei is a rare species. It is distributed within the Yangtze River basin, the Henan Nanling Mountains, and several mountains and valleys in Shaanxi, Gansu, and also in the Taiwan Province in China (Liet al., 2007). It has attracted wide attention because of the significant anticancer activity of taxol present in bark, twigs, leaves and other parts of the species(Soniaet *al.*, 2011,Elavarasiet *al.*, 2012, Zhaoet *al.*, 2007, Ozols, 1995, Shaoet al., 2012, Li et al., 2008).The species has faced huge harvesting pressure in recent years and populations have been decimated. Thus, researchers have been seeking ways to protect*T. chinensisv. mairei* populations within a framework of forest resource management and genetic conservation. In addition, seeds of this species have a combination of morpho-physiological deep dormancy with underdeveloped, dormant embryos, which need grow to a certain length before seed dormancy could be broken. Its natural reproduction is low, resulting in an endangered existing state of this species. The taxolactive ingredient of *T chinensisv. mairei*.is reported to have significant inhibitive effects on ovarian cancer, breast, lung, stomach, colon, melanoma, leukemia, bladder cancer, and central nervous system tumors(Yuan,*et al.*,2002a, 2002b, 2002c, Li,et a1.,2003, Kingstone*t al.*, 1993, Kumaran*et al.*, 2010). At present, the taxanes and non-taxanes compounds were isolated from *T. mairei*(Bergstralhet al., 2006, Yang*et al.*, 2011, Yanget al., 2012). The polysaccharide compounds of this species also can improve immunity and protect the liver and other organs(Li*et al.*,2007). *T. chinensisv.mairei* was listed as one of China's class key protected wild plants in 1999.

In this paper, extracts of petroleum ether, ethyl ether, ethyl acetate and methanol of *T. maireis* per moderm and endosperm were identified by GC-MSto provide reference for further development and utilization of this species.

## Materials and Methods

### Experimental materials

*T. chinensisv.mairei* fruits consist of scarlet or green cuplike arils. Fruits were collected from 20-40 years old trees in anmixed broad-leaved forest in valleys and slopes at 400-500m above sea level in 2011, Xiushui County Jiujiang City, Jiangxi Province. Arils and empty seedswere floated off after fruits were collected and macerated in water. The natural dried seeds were sealed into polyethylene bags and stored in the refrigerator (4°C). The TGW of seeds was 65.048g

### Experimental methods

#### *Sample Extraction and Separation*

Spermoderm and endosperm (including embryos) of *T. chinensisv.*

*mairei* seeds were separated and weighed. Spermoderm and endosperm (1200g) were separately placed into two 1000 ml beaker after they were grind with a mill. Afterwards the beakers were filled with methanol (80%) and closed for extraction at 0 to 4 °C. The extract was then filtered by Büchner funnel at intervals of 24h. The collected filtrate was re-extracted with 80% methanol. The process was repeated several times until the extract turned pale, and all extracts were mixed. The methanol extracts of *T. chinensisv.mairei* spermoderm and endosperm were crudely isolated with system solvent(Fig.1, Zuet al. 2010). The crude extracts were separated into four groups: petroleum ether phase, ether phase, ethyl acetate phase and methanol phase. And then, these organic phases of spermoderm and endosperm were placed in a Rotary Evaporator RE-3000 concentrates to evaporate. Finally, the organic phases were concentrated to 200ml and placed at 5 °C.

**Identification of extractions of *T. chinensis v. maireis* permoderm and endosperm**

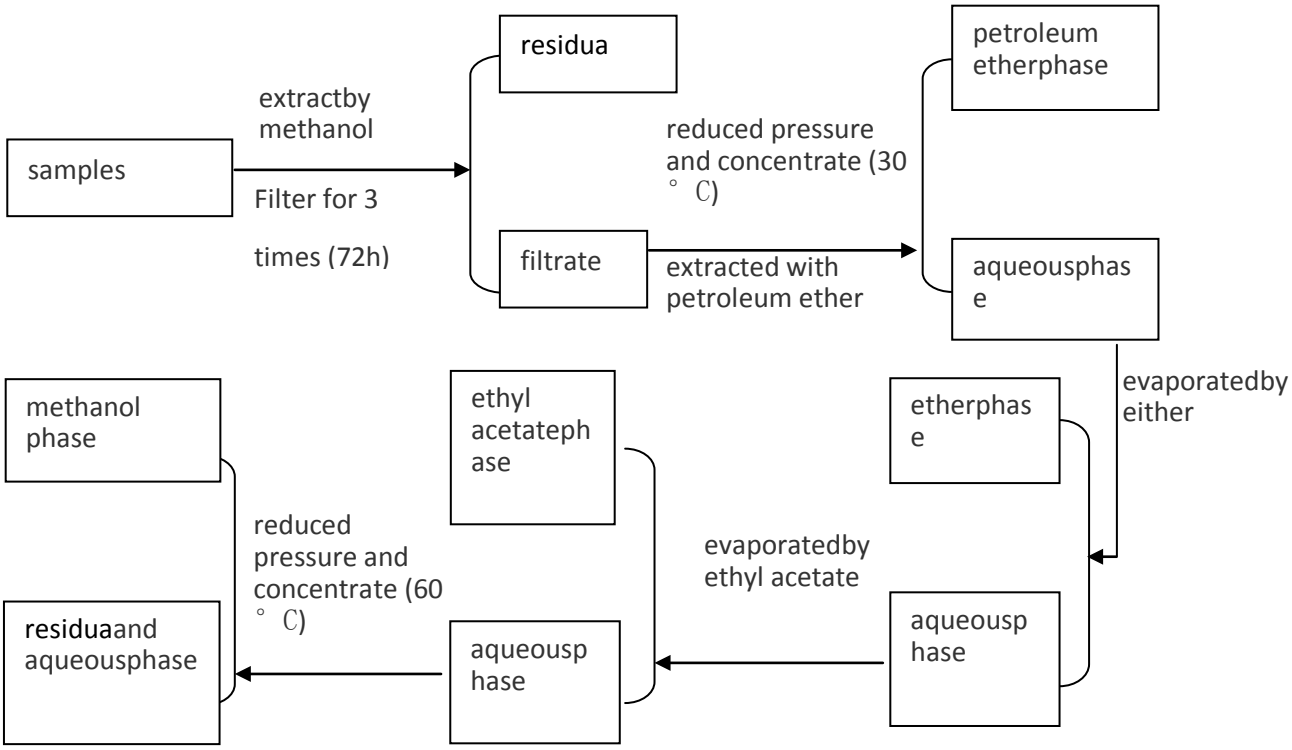
Each above 100 ml concentrated extract was collected and evaporated to be concentrated dry in vacuum on a rotary evaporator. These dry matters were respectively washed by the same organic solvent to obtain 3ml samples. The samples were identified by GC-MS in the Forest Products Chemistry, Analysis and Testing Center of Chinese Academy of Forestry.

Analysis was carried out on a GC-MS instrument (Antigone Lun 6890N/5973N, USA) equipped with a 1NNOWAX quartz capillary column (30m×0.25mm; film thickness 0.25 μm) with 50 °C-190°C column temperature; programmed temperature was 5°C · $min^{-1}$; Helium was used as a carrier gas; gasification temperature was 280°C. MS conditions were: The ionization mode was EI and the ionization energy was 70eV; source temperature was 200°C; collection of current was 300μA; emission current was 1mA; instrument separation rate was 600; quality range was 10-500. Finally, spectrums of all components were checked by the inventory signal of computer-controlled and matched with the standard spectrum(Zu*et al.*,2010).

## Results

The peaksidentified by GC-MS showed that there were a variety of organic compounds in *T. chinensisv. maireis*permoderm and endosperm (Fig. 2). These compounds were mainly organic acids,

**Fig. 1 Theisolating process of methanol extracts with different solvents of *Taxuschinensisvar* mairei spermoderm and endosperm**




benzene and ester-based. 24 kinds of organic compounds were identified in *T. chinensisv. maireis*permoderm. In addition, there were 4 indefinite and 9 unknown substances in spermoderm as shown in the table 1. Petroleum ether phase of spermoderm included (Z) -6 - octadecenoic acid (32.53%), unknown 1 (31.28%), erucic acid (19.26%), and hexadecanoic acid (7.14%); ether phase mainly included 9-18 carbon acid (43.27%), hexadecanoic acid (15.05%), erucic acid (11.12%), oleic acid (10.11%), ether (6.46%), octadecanoic acid (5.16%); ethyl acetate phase of spermoderm mainly included 9 - octadecadienoic acid (44.15%), hexadecanoic acid (35.85%), octadecanoic acid (8.06%), 3 - phenyl -2 - acrylic acid (6.60%); methanol phase of spermoderm mainly included erucic acid (65.09%) 9 - 18 carbon acid (7.83%), unknown 4 (4.03%), oil acid (3.79%), and so on. 37 organic compounds were identified in *T. chinensisv. mairei* endosperm, which included 5 types unknown chemicals. Endosperm petroleum ether phase mainly included organic compounds, (E) -9 - octadecenoic acid (15.32%), unknown 5 (12.31%), unknown 6 (6.69%), unknown 7 (14.10%), 9 - octadecadienoic acid (6.40%), erucic acid (6.38%); ether phase mainly included erucic acid (72.25%), oleic acid (12.42%), cis-9 ,10-epoxyoctadecanoic acid (8.88%); ethyl acetate phase mainly included (Z, Z) -9,12 - 18 oleic acid (45.70%), 3 - phenyl -2 - acrylic acid (10.48%), 20 acid (10.31%), oleic acid (8.13%), erucic acid (7.21%), hexadecanoic acid (4.55%); methanol phase mainly included diethyl phthalate (38.79%) , (2,3) - dihydro--1,1,3 - trimethyl -3 - phenyl-1H-indene (22.42%), 1,2,3,4 - tetracarboxylic benzene (8.50%), 2 , 4 - 2-phenyl -4 - methyl--2 (E) - pentene (5.22%), as shown in table 1.

**Table.1 Relative contents indifferent extractsof *Taxuschinensisvar*.maireiseeds**

| No. | Compositions | molecular formula | Contents % | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | petroleum ether extract | | methanolic extract | | ethyl acetate extract | | ether extract | |
| | | | endosperm | spermoderm | endosperm | spermoderm | endosperm | spermoderm | endosperm | spermoderm |
| 1 | (2,3)- dihydro -1,1,3-trimethyl -3- phenyl -1H-indene | $C_{18}H_20$ | | | 22.42 | | | | | |
| 2 | (E)-9- octadecenoic acid | $C_{18}H_{34}O_2$ | 15.32 | | | | 3.19 | | | |
| 3 | (Z)-6- octadecenoic acid | $C_{18}H_{34}O_2$ | | 32.53 | | | | | | |
| 4 | (Z,Z)-9,12-octadecadienoic acid | $C_{18}H_{32}O_2$ | | | | | | | 0.49 | |
| 5 | (Z,Z)-9,12-oleic acid | $C_{18}H_{32}O_2$ | 9.46 | 2.47 | | | 45.7 | 3.12 | | |
| 6 | 1,2,3,4-tetramethylbenzene | $C_{10}H_{14}$ | | | 8.5 | | | | | |
| 7 | 18- jecoleic acid | $C_{19}H_{36}O_2$ | | | | | | | | 0.74 |
| 8 | 1-ethide-2,3-dimethyl benzene | $C_{10}H_{14}$ | | | 0.15 | | | | | |
| 9 | 2,4-diphenyl-4-methyl-2(E)-pentene | $C_{18}H_20$ | | | 5.22 | | | | | |
| 10 | 3- phenyl -2-acrylic acid | $C_9H_8O_2$ | | | | | 10.48 | 6.6 | 2.21 | 0.97 |
| 11 | 9- octadecenoic acid | $C_{18}H_{34}O_2$ | 6.4 | 4.36 | | 7.83 | | 44.15 | | 45.45 |
| 12 | phenylformic acid | $C_7H_6O_2$ | | | | | 1.3 | | 1.62 | |
| 13 | cluytyl alcohol; octacosyl alcohol | $C_{28}H_{58}O$ | | | | 1.27 | | | | |
| 14 | eicosanoic acid | $C_{20}H_{40}O_2$ | | | | | 10.31 | | | 2.1 |
| 15 | caproic acid | $C_6H_{12}O_2$ | | | 1.74 | | | | | |
| 16 | erucic acid | $C_{22}H_{42}O_2$ | 6.38 | | | 65.09 | 7.211 | | 72.25 | 11.12 |
| 17 | diethyl phthalate | $C_{12}H_{14}O_4$ | | | 38.79 | | | | | |
| 18 | pelargonic acid | $C_9H_{18}O_2$ | | | 2.43 | | | | | |
| 19 | stearic acid | $C_{18}H_{36}O_2$ | 4.59 | | | | | 8.06 | | 5.16 |
| 20 | hexadecanoic acid | $C_{16}H_{32}O_2$ | 3.58 | 7.14 | | 0.76 | 4.55 | 35.85 | 0.75 | 15.05 |
| 21 | octanoic acid | $C_8H_{16}O_2$ | | | 3.48 | | | | | |
| 22 | acetic acid | $C_2H_4O_2$ | | | | | | | | 6.46 |
| 23 | oleic acid | $C_{18}H_{34}O_2$ | | | | 3.79 | 8.13 | | 12.42 | 10.11 |
| 24 | n-Heptanoic acid | $C_7H_{14}O_2$ | | | 4.03 | | | | | |

## Conclusion and Discussion

56 peaks of *T. maireis*permoderm components and 79 peaks of endosperm were obtained by GC-MS. 37 chemicals were detected by computer on-line information retrieval, 24 of which compounds were identified (64.86%), and there were other 4 indefinite kinds (10.81%) and 9 unknown kinds (24.32%). 35 compounds with content more than 1%, accounted for 94.59%of the total extracted compounds. 32 peaks were obtained in petroleum ether extractions, 7 of which were identified (21.88%), and their content were more than 1%. 36 peaks were obtained in methanol extractions, 14 of which were identified (38.89%), and 13 compounds content more than 1%, accounting for 92.86% of the total of all compounds. 34 peaks of ethyl acetate extractions were obtained and of which 10 compounds (29.41%) were identified, 13 of which compounds content were more than 1%, accounting for 92.86% of the total of all compounds. 33 peaks of ether extracts were obtained, 11 of which compounds (33.33%) were identified, 2 kinds of components had indefinite substances (6%). Their relative content of components was identified with the peak area normalization method.The results also showed more kinds of organic compounds were extracted from *T. chinensisv. mairei* in endosperm than those in spermoderm on the same treatment condition. The same organic compounds might exist in the different organic phase of spermoderm and endosperm, such as erucic acid also existed in petroleum ether phase, ether phase, methanol phase of spermoderm, and petroleum ether phase, ether phase and ethyl acetate phase of endosperm phase. 16 acids existed in petroleum ether, ethyl ether phase, ethyl acetate phase of endosperm and methanol phase of spermoderm.

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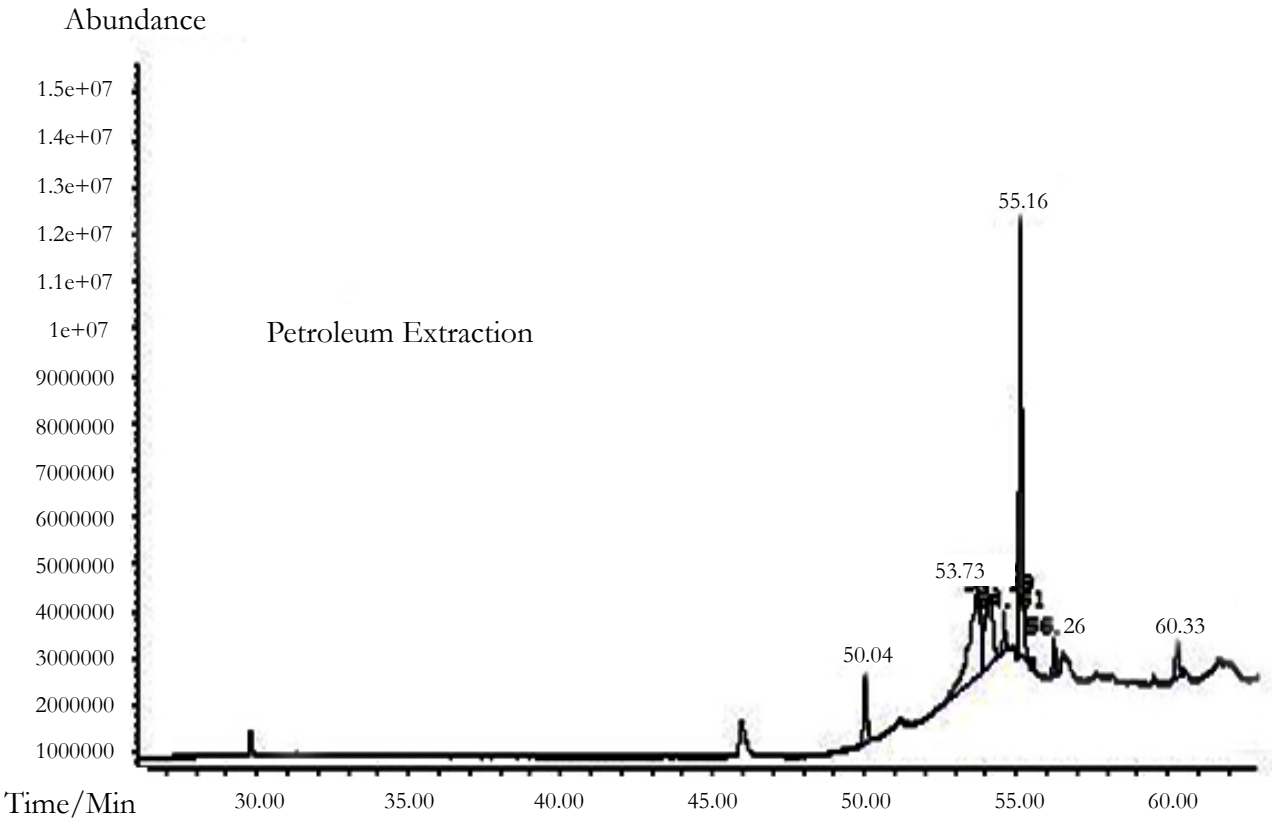
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1.2e+07
1.1e+07
1e+07
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8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
Petroleum Extraction
55.16
53.73
56.26
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50.04
Time/Min
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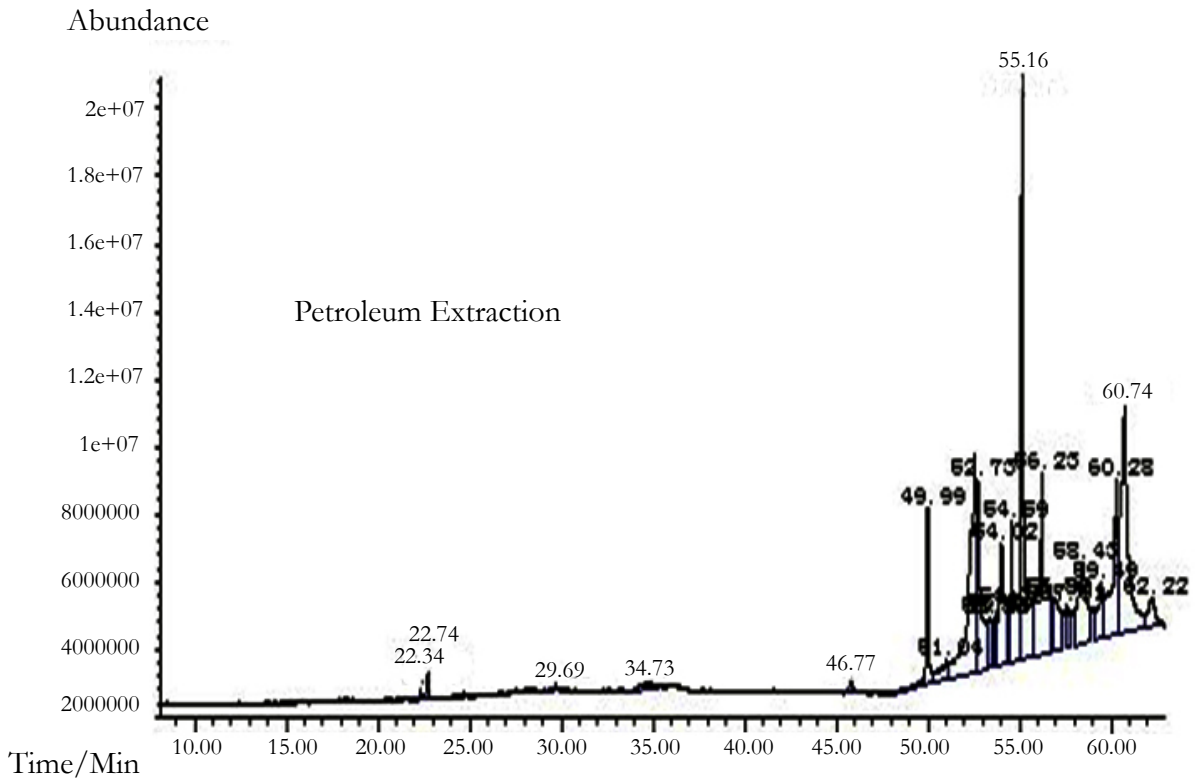
35.00
40.00
45.00
50.00
55.00
60.00

GRPH 2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Petroleum Extraction
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49.99
22.74
22.34
29.69
34.73
46.77
Time/Min
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15.00
20.00
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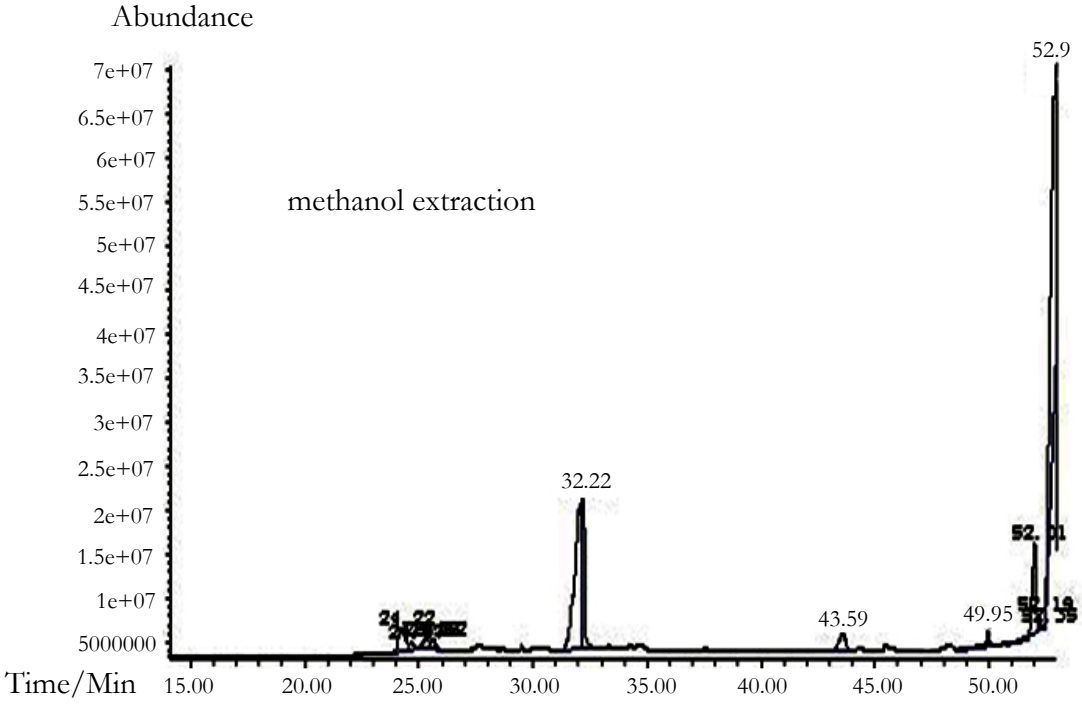
40.00
45.00
50.00
55.00
60.00

GRPH 3


Abundance
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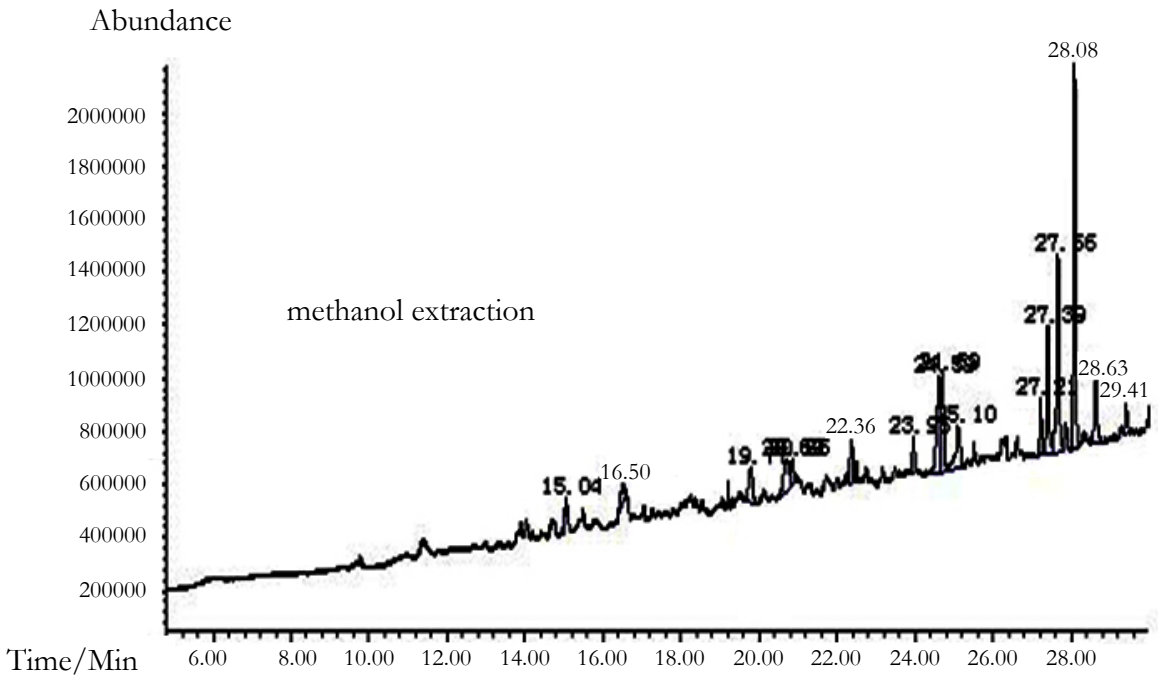
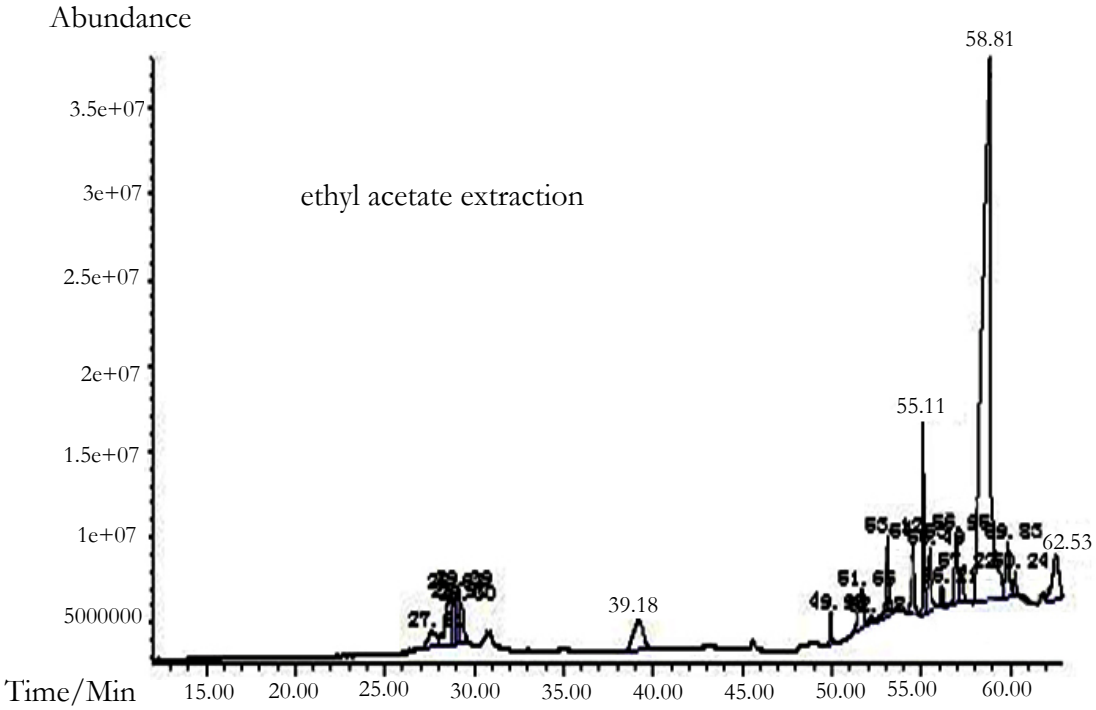
52.9
32.22
43.59
49.95
Time/Min

GRPH 4


Abundance
methanol extraction
28.08
28.63
29.41
22.36
16.50
Time/Min

GRPH 5


Abundance
ethyl acetate extraction
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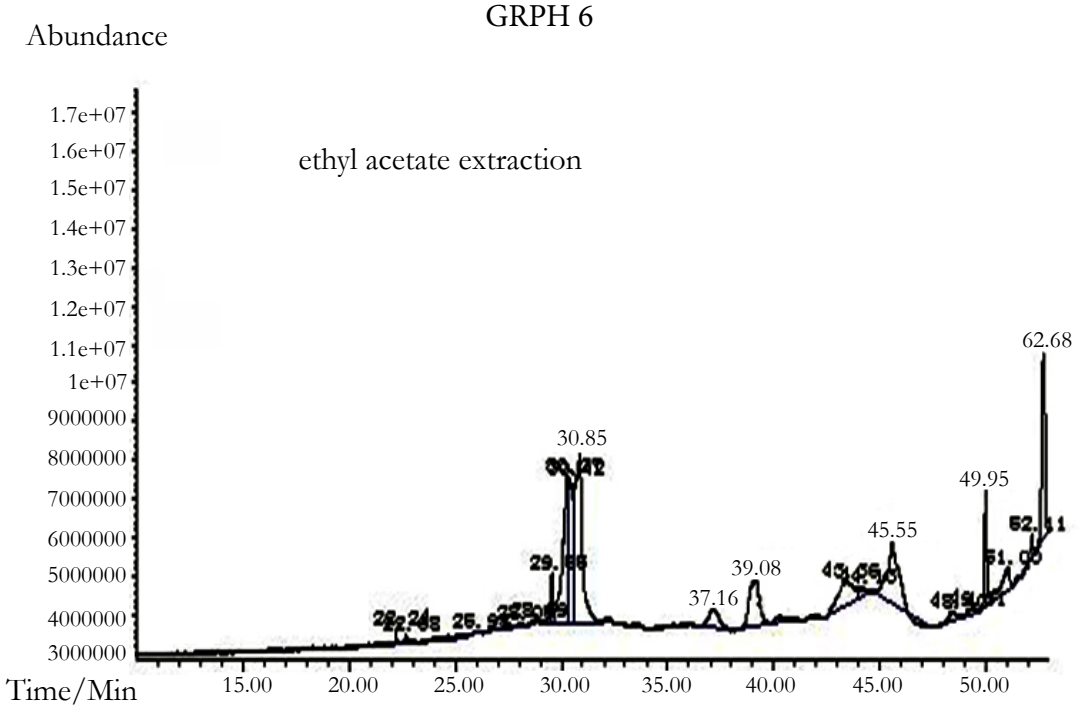
3e+07
2.5e+07
2e+07
1.5e+07
1e+07
5000000
Time/Min
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00

GRPH 6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30.85
49.95
45.55
39.08
37.16
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1.6e+07
1.5e+07
1.4e+07
1.3e+07
1.2e+07
1.1e+07
1e+07
9000000
8000000
7000000
6000000
5000000
4000000
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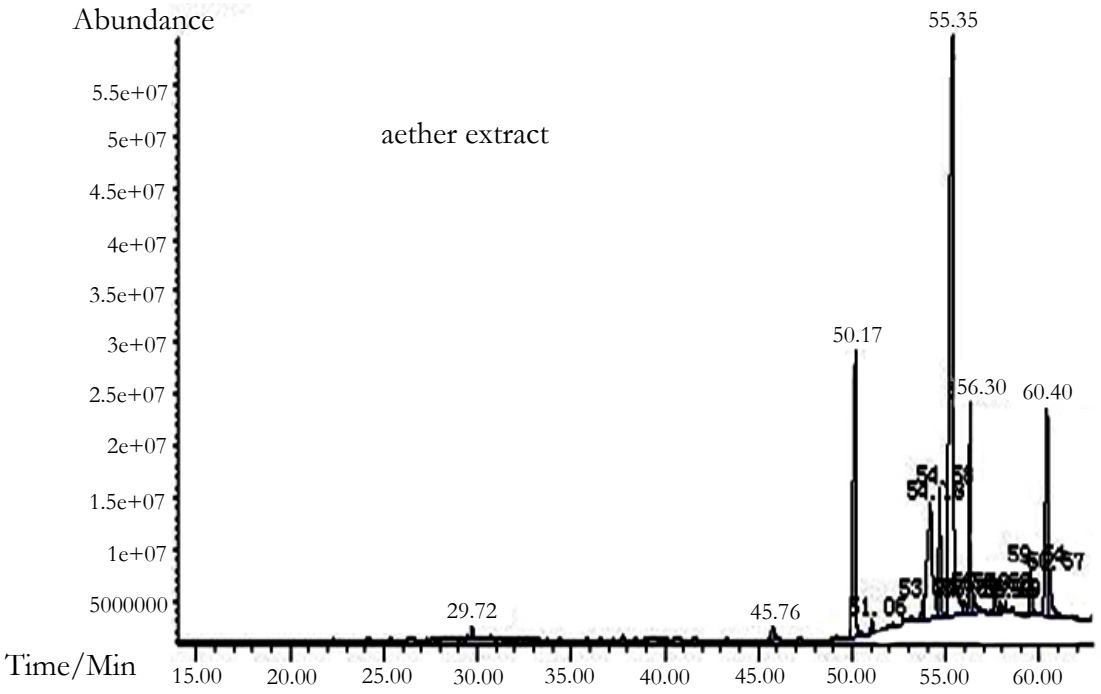
35.00
40.00
45.00
50.00

GRPH 7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2.5e+07
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1e+07
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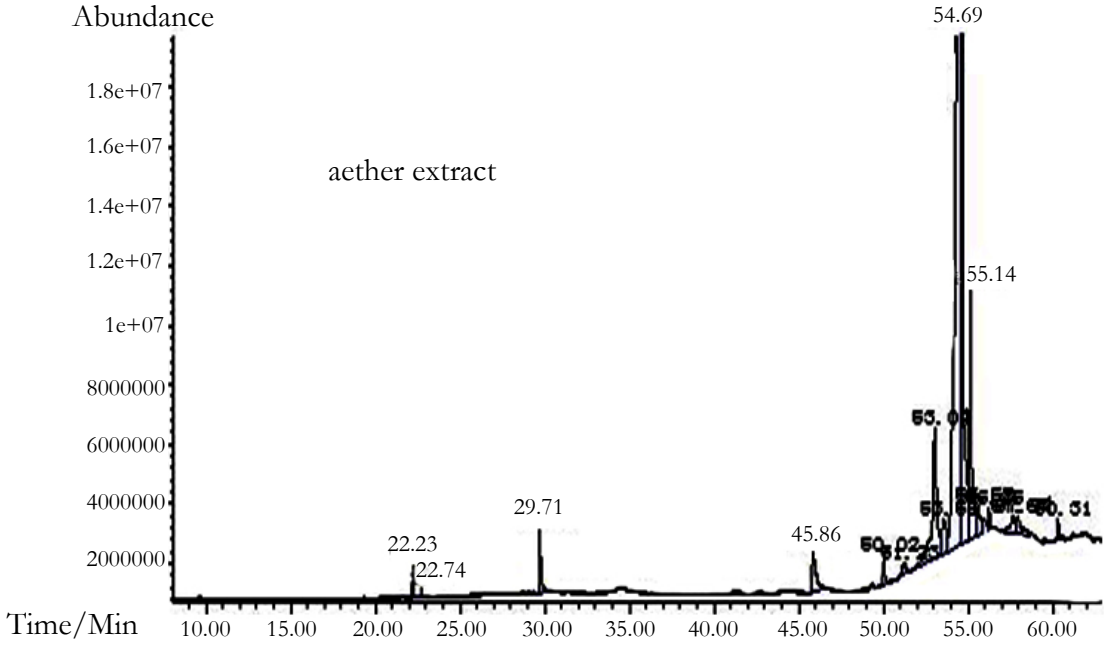
50.00
55.00
60.00

GRPH 8


Abundance
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22.23
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45.86
1.8e+07
1.6e+07
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1.2e+07
1e+07
8000000
6000000
4000000
2000000
Time/Min
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00

In recent years, researchers have made deep study of *T. chinensisv. mairei*, but reports on artificial propagation and cultivation of *T. mairei* research were rare(Shi*et al*., 2010, Yu*et al*., 2012a, 2012b). Because research on suitable germination conditions was rudimentary, identifying chemical components and its effects on seed germination is important for protection and cultivation of this species. The development and utilization of *T. chinensisv. maire* were seriously affected by its low seed germination rate(Yang *et al*., 2012). As a rare plant species,T. chinensisv. mairei should be vigorously cultivated on precondition of ensuring the quality of medicines, so that their medicinal value can be sustainable and developed.

## Acknowledgements

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## References

[1]. Bergstralh, D.T., Ting, J.P. 2006. Microtubule stabilizing agents: their molecular signaling consequences and the potential for enhancement by drug combination. Cancer Treat. Rev, 32, 166-179.

[2]. Elavarasi A, Rathna G S, Kalaiselvam M. 2012. Taxol producing mangrove endophytic fungi Fusarium oxysporum from Rhizophora annamalayana. Asian Pacific Journal of Tropical Biomedicine, S1081-S1085.

[3]. Kingston, D C., Molinero A A., Rimoldi, J. M. et al.. 1993. Progress in the chemistry of organic natural products studies. Vienna: Springer, 61, 1-206.

[4]. Kumaran R S. Kim H J, Hur B K. 2010. Taxol promising fungal endophyte, Pestalotiopsis species isolated from Taxus cuspidate. Journal of Bioscience and Bioengineering, 110(5),541-546.

[5]. Li C, Yuan Y J, Wu J C, et a1.. 2003. A structured kinetic model for suspension of Taxus chinensis var. mairei induced by an oligosaccharide from Frusarium oxysporum Biotechnology Letters, 25, l335-1343.

[6]. Li C F, Liu Y, Dong M, Shi Q W. 2007. Advances in studies on chemical constituents in Taxus chinensis var. mairei. Chin Tradit Herb Drugs,38:1121-1132.

[7]. Li C F, Huo C H, Zhang M L, et al.. 2008. Chemistry of Chinese yew, Taxus chinensis var. mairei. Biochemical Systematics and Ecology, 36:266-282.

[8]. Ozols R F. 1995. USA update on pacilitaxl in ovarian cancer. Ann Med., 27, 127-128.

[9]. Shao N, Wang S M, Yao C, et al.. 2012. Sequential versus concurrent anthracyclines and taxanes as adjuvant chemotherapy of early breast cancer: A meta-analysis of phase III randomized control trials. The Breast, 21:389-393.

[10]. Shi G L, Bai B, Lu C H. 2010. Seed rain and seed bank of Chinese yew (Taxus chinensis var. mairei) population in Tianmu Mountain. Acta Ecologica Sinica 30, 276-279.

[11]. Sonia M, Rosa M C, Mohammad H M, et al. 2011. Production of the anticancer drug taxol in Taxus baccata suspension cultures: A review. Process Biochemistry 46,23-34.

[12]. Yang C, Wang J S, Kong L Y. 2011. Chemical Constituents from the Needles of Taxus canadensis. Chinese Journal of Natural Medicines, 9(2), 0188-0190.

[13]. Yang W X, Zhao Z G, Wang L H, et al.. 2012. Control of hypertension in rats using volatile components of leaves of Taxus chinensis var. mairei. Journal of Ethnopharmacology, 141,309-313.

[14]. Han B R, Huang Y G, Li X G, et. al.. 2000. Six kinds germination inhibitors in American ginseng pulp. Special Wild Economic Animal and Plant Research, 1, 13-17.

[15]. Yuan Y J, Li C, Hu Z D, et al.. 2002a. A double oxidative burst for taxol production in suspension cultures of Taxus chinensis var. mairei induced by oligosaccharide from Fusarium oxysprum. Enzyme and Microbial Technology, 30, 774-778.

[16]. Yuan Y J, Li C, Hu Z D, et al.. 2002b. Fungal elicitor-induced cell apoptosis in suspension cultures of Taxus chinensis vat. mairei for Taxol production. Process Biochemistry, 38, 193-198.

[17]. Yuan Y J, Li C, Wu J C, et al. 2002c. A model for signal transduction in suspension cultures of Taxus chinensis var. mairei induced by an oligosaccharide from Fusarium oxysporum. Biotechnology Letters, 24, 407-412.

[18]. Yu J H, Wang Y B, Qian H. 2012a. Polyprenols from the needles of Taxus chinensis var. mairei. Fitoterapia, 83,831–837.

[19]. Yu J H, Wang Y B, Qian H, et al.. 2012b. Polyprenols from Taxus chinensis var. mairei prevent the development of CCl4-induced liver fibrosis in rats. Journal of Ethnopharmacology 142,151-160.

[20]. Zhao D, Qin K, Cao C M, et al.. 2007. Effect of the compounds from Taxus chinensis var. mairei and T. cuspidata on the proliferation of human breast cancer cells. Natural Product Research and Development, 4, 635-638.

[21]. Zu Y G, Pang H H, Yu J H, et al.. 2010. Responses in the morphology, physiology and biochemistry of Taxus chinensis var. mairei grown under supplementary UV-B radiation. Journal of Photochemistry and Photobiology B: Biology 98,152-158.