GC-MS Analysis of Chemical Components of \textit{Taxus Chinensis} Var. Mairei Seeds

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Abstract

The extracts of petroleum ether, methanol, ethyl acetate and ether from \textit{Taxus chinensis} var. \textit{mairei} seeds were analyzed by GC-MS, and relative contents were determined using a normalized method. 56 peaks were obtained from extracts of endosperm of \textit{T. chinensis} var. \textit{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%). \textit{T. chinensis} var. \textit{mairei} seeds have various bio-active ingredients and a higher value for development and utilization as a medicinal plant.

Keywords: \textit{Taxus chinensis} var. \textit{mairei}; seed; chemical components; GC-MS; composition analysis

Introduction

\textit{T. chinensis} var. \textit{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(Soniet al., 2011, Elavarasi et al., 2012, Zhao et al., 2007, Ozols, 1995, Shao et 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 \textit{T. chinensis} var. \textit{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 taxol active ingredient of \textit{T. chinensis} var. \textit{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 al., 2003, Kingstomet al., 1993, Kumaraemet al., 2010). At present, the taxanes and non-taxanes compounds were isolated from \textit{T. mairei}(Bergstralhet al., 2006, Yang et al., 2011, Yang et al., 2012). The polysaccharide compounds of this species also can improve immunity and protect the liver and other organs(Liet al., 2007). \textit{T. chinensis} var. \textit{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 \textit{T. mairei} per modern and endosperm were identified by GC-MSto provide reference for further development and utilization of this species.

Materials and Methods

Experimental materials

\textit{T. chinensis} var. \textit{mairei} fruits consist of scarlet or green cuplike arils. Fruits were collected from 20-40 years old trees in annexed broad-leaved forest in valleys and slopes at 400-500m above sea level in 2011, Xiushui County Jiujiang City, Jiangxi Province. Arils and empty seeds were 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.
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. chinensis var. mairei spermoderm and endosperm were crudely isolated with system solvent(Fig1, Zue et 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 var. mairei spermoderm 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 1NNOX 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(Zue et al., 2010).

Results

The peaks identified by GC-MS showed that there were a variety of organic compounds in T. chinensis var. maireispermoderm and endosperm (Fig. 2). These compounds were mainly organic acids, benzene and ester-based. 24 kinds of organic compounds were identified in T. chinensis var. maireispermoderm. 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 (7.83%), unknown 1 (31.28%), unknown 2 (8.13%), erucic acid (7.21%), hexadecanoic acid (4.55%); ether phase mainly included erucic acid (72.25%), oleic acid (14.10%), 9-octadecadienoic acid (6.40%), erucic acid (6.38%); ethyl acetate phase mainly included (Z, Z)-9,12 -18 oleic acid (45.70%), 3 -phenyl -2 - acrylic 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%); 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.
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Conclusion and Discussion

56 peaks of T. mairei spermoderm 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 compounds 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. chinensis v. 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.

| No. | Compositions                                      | molecular formula | Contents % |
|-----|---------------------------------------------------|-------------------|------------|
|     |                                                   | en-dosperm        | sperm-derm | en-dosperm | sperm-derm | endosperm | spermoderm | endosperm | spermoderm |
| 1   | (2,3)- dihydro -1,1,3-trimethyl 5- phenyl -1H-indene | C9H10              |            |            | 22.42      | 3.19       |
| 2   | (E)-9- octadecenoic acid                          | C18H34O2           | 15.32      | 3.19       |
| 3   | (Z)-6- octadecenoic acid                          | C18H34O2           | 32.53      | 3.19       |
| 4   | (Z,Z)-9,12-octadecadienoic acid                   | C18H32O2           | 0.49       |
| 5   | (Z,Z)-9,12-oleic acid                            | C18H32O2           | 9.46       | 2.47       | 45.7       | 3.12       |
| 6   | 1,2,3,4-tetramethylbenzene                        | C10H14             | 8.5        |
| 7   | (E)-octadecenoic acid                             | C18H34O2           | 0.15       |
| 8   | 2,4-diphenyl-4-methyl-2-(E)-pentene               | C12H10             | 5.22       |
| 9   | (E)-3-phenyl -2-acrylic acid                      | C12H10             | 10.48      | 6.6        | 2.21       | 0.97       |
| 10  | 9- octadecenoic acid                              | C18H34O2           | 6.4        | 4.36       | 7.83       | 44.15      | 45.45      |
| 11  | phenylformic acid                                 | C7H6O2             | 1.3        | 1.62       |
| 12  | phenylformic acid                                 | C7H6O2             | 1.7        |
| 13  | 2,3 dihydro -1,3-dimethyl benzene                 | C8H10              | 1.27       |
| 14  | n-Heptanoic acid                                  | C7H14O2            | 10.31      | 2.1        |
| 15  | caproic acid                                      | C6H12O2            | 1.74       |
| 16  | diethyl phthalate                                 | C12H10             | 6.38       | 65.09      | 7.211      | 72.25      | 11.12      |
| 17  | diethyl phthalate                                 | C12H10             | 38.79      |
| 18  | pelargonic acid                                    | C10H18O2           | 2.43       |
| 19  | stearic acid                                      | C18H36O2           | 4.59       | 8.06       | 5.16       |
| 20  | hexadecanoic acid                                 | C16H32O2           | 3.58       | 7.14       | 0.76       | 4.55       | 35.83      | 0.75       | 15.05      |
| 21  | octanoic acid                                     | C8H16O2            | 3.48       |
| 22  | octanoic acid                                     | C8H16O2            | 3.48       |
| 23  | oleic acid                                        | C18H36O2           | 3.79       | 8.13       | 12.42      | 10.11      |
| 24  | n-Heptanoic acid                                  | C7H14O2            | 4.03       |

Table 1: Relative contents indifferent extracts of Taxus chinensis var. mairei seeds

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**GRAPH 3**

- Abundance
- Time/Min: 15.00, 20.00, 25.00, 30.00, 35.00, 40.00, 45.00, 50.00
- Methanol extraction

**GRAPH 4**

- Abundance
- Time/Min: 6.00, 8.00, 10.0, 12.00, 14.00, 16.00, 18.00, 22.00, 24.00, 26.00, 28.00
- Methanol extraction
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Gao H, Zhang Y, LU ShunBao. (2013). GC-MS Analysis of Chemical Components of Taxus Chinensis Var. Mairei Seeds. Int J Microbiol Adv Immunol. 1(2), 6–13.
In recent years, researchers have made deep study of *T. chinensis var. 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. chinensis var. mairei* were seriously affected by its low seed germination rate (Yang et al., 2012). As a rare plant species, *T. chinensis var. mairei* should be vigorously cultivated on precondition of ensuring the quality of medicines, so that their medicinal value can be sustainable and developed.

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