

Full Length Research Paper

# Aliphatic aldehyde rich volatile constituents of *Houttuynia cordata* from southwest China

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*Houttuynia cordata* is an aromatic herb with great importance in the food, pharmaceutical and fragrance industries. Previous studies have shown that its essential oil mainly contains two types of compounds, terpenes and aldehydes. The essential oil composition of a new accession from southwest China was investigated. Head-space solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) analyses led to the identification of 13 compounds, representing 98.79% of the volatile constituents. Aliphatic aldehydes (69.64%) were quantified as the principal class of the components besides acids (22.83%). However, no terpenoids were detected. The essential oil was dominated by decanal (42.64%). Decanoic acid (18.67%) and dodecanal (15.04%) were also the major constituents. Comparison of results with previous reports classified the current accession as a new chemotype rich in aliphatic aldehydes and acids, with complete absence of regular mono- and sesquiterpenoids.

**Key words:** Essential oil, *Houttuynia cordata*, terpenes.

## INTRODUCTION

*Houttuynia cordata* Thunb. is an aromatic herb native to many Asian countries (Chakraborti et al., 2006; Nuengchamngong et al., 2009; Ogle et al., 2003; Wu et al., 2005a). It is widely distributed in ravines, streamsides, forests, wet meadows, slopes, thicket and field margins, trailsides, roadsides or ditch banks in these regions (Xu et al., 2011). In China it is known as 'Yuxingcao', which means 'producing unique fishy smell'. Its young plants are popularly used as wild vegetable and its plants are generally used as traditional Chinese medicine (TCM) for hundreds of years. Many studies have demonstrated that it processes a variety of pharmacological activities such as anticancer, antioxidation, antiviral, antibacterial and antiallergic and is effective in treating pneumonia, influenza, severe acute respiratory syndrome (SARS) and human immunodeficiency virus (HIV) (Han et al., 2009; Hayashi et al., 1995; Kim et al., 2001; Lau et al.,

2008; Toda, 2005). It therefore has been identified as one of the most potential medicinal and edible wild plant resources in China by the Chinese State Health Department (Wu et al., 2005b).

The essential oil of *H. cordata* is one of the major effective components (Nuengchamngong et al., 2009). Previous studies have shown that the essential oil are dominated by two types of compounds, terpenes and aldehydes (Chen et al., 2008). However, *Houttuynia* derives from the ancient Saururaceae family and the current *H. cordata* is the relic of its agamic race (Liang, 1995). It appears that heterozygosity, polyploidy and aneuploidy through inter-specific hybridization and cytotoxicity, and *H. cordata* populations exhibit highly variations in chromosome number and at the nuclear genome level (Wu et al., 2003, 2005a,b,c). The composition of the essential oil therefore represents significant variations among *H. cordata* accessions (Chen et al., 2008).

Previous studies reported that terpenoids could be observed in nearly all *H. cordata* accessions mainly collected from Sichuan and Chongqing provinces in China according to the analyses of the essential oil. In general, two chemotypes, myrcene (M) and decanal (D) chemotypes, could be described within the investigated

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*H. cordata* accessions from China (Chen et al., 2008). Chemotype M is rich in terpenes dominated by myrcene and monoterpenoids which are the main class of volatile components, followed by a minor share of sesquiterpenoids. Chemotype D contains abundant aldehydes dominated by decanal and has a lower level of terpenes. However, large genetic diversity has been observed among *H. cordata* populations, the germplasm resource with the absence of terpenes in the essential oil is not available until now. In the present study, we attempt to report the essential oil composition of a new line of *H. cordata*, of which terpenoids are absent from the essential oil.

## MATERIALS AND METHODS

### Plant materials and growth conditions

*H. cordata* new line (accession No.: x10-1) was collected from Yucheng District, Ya'an, southwest China. Its habitat is roadside and wild. The voucher specimen is deposited in Sichuan Agricultural University, Ya'an, China. *H. cordata* w01-100 belonging to chemotype M was used as control in the present study (Chen et al., 2008; Wu et al., 2003). They were planted in the farm of Sichuan Agricultural University, Ya'an, China (latitude 29° 59' 08"N, longitude 102° 58' 56"E, and altitude 595 m). In October 2010, leaves were collected randomly and used for determination of essential oil.

### Essential oil extraction

The essential oil was prepared by headspace solid-phase microextraction (HS-SPME) (Liang et al., 2005). The manual SPME holder was used with a 100 µm polydimethylsiloxane fibre assembly (Supelco, Bellefonte, USA). The fibre was conditioned as recommended by the manufacturer. The sample (0.5 g) was hermetically sealed in a 4 ml vial. The fibre was suspended in the headspace and equilibrated for 20 min in a thermostatic bath at 90°C.

### GC-MS analysis

The compositional analysis of the volatile constituents extracted by Head-space solid phase microextraction (HS-SPME) was carried out by a GC (Agilent Technologies 6890N) interfaced with a mass selective detector (Agilent 5973B) equipped with an Agilent HP-5MS silica capillary column (30 m × 0.25 mm and film 0.25 µm). The carrier gas was helium with a constant flow rate of 1 ml min<sup>-1</sup>. Injector temperatures were 250°C. A split injection with a ratio of 1:20 was used.

The oven temperature was set at 60°C for 2 min, programmed to 110°C at a rate of 10°C min<sup>-1</sup>, and held at 110°C for 4 min, then heated to 220°C at a rate of 10°C min<sup>-1</sup>, isothermal at this temperature for 4 min. The mass spectra operating parameters were as follows: ionisation potential, 70 eV; interface temperature, 280°C. Relative percentage of volatile components was evaluated from the total peak area by apparatus software. Identification of components was based on the comparison of their mass spectra and retention time with those of authentic compounds and by computer matching with NIST 2.0 and Wiley libraries as well as by comparison of the mass spectral data with those reported in the authentic references (Chen et al., 2008).

## RESULTS AND DISCUSSION

In order to ensure the accuracy of measurement, a reference accession, *H. cordata* w01-100, was parallelly used as control in the whole experimentation. The HS-SPME and GC-MS analyses of the leaf essential oil of *H. cordata* w01-100 led to the identification of 30 compounds, of which 15 terpenes were identified, accounting for 48.69% of the essential oil (Table 1). Monoterpenoids (46.85%) were the main class of essential oil components, which were dominated by monoterpene hydrocarbons (41.90%), and followed by a minor share of sesquiterpenoids (1.84%). The chemical composition is in accordance with the chemotype principles of *H. cordata* w01-100 (Chen et al., 2008) and the method for determination of essential oil proved to be accurate in the present study.

Result showed the presence of 13 compounds in the leaf essential oil of *H. cordata* x10-1, representing 98.79% of total oil (Table 1). Interestingly, no terpenes were detected in *H. cordata* x10-1. In general, aldehydes (69.64%) were quantified as the principal class of components and beyond them were placed the acids (22.83%). Alcohols (5.96%) had a minor share in the essential oil, and esters (0.37%) had only a small portion percentage. Contrary to *H. cordata* w01-100, *H. cordata* x10-1 did not generate ketones. Decanal (42.64%) was the major component of the essential oil of *H. cordata* x10-1. Decanoic acid (18.67%) and dodecanal (15.04%) were also identified as the major constituents in the essential oil, along with nonanal, 1-nonanol, 1-decanol, undecanal, undecanoic acid, tridecanal, dodecanoic acid, tetradecanal, hexadecanal, and decanoic acid, decyl ester as minor constituents. According to the chemical profile of the essential oil, *H. cordata* x10-1 should be classified into chemotype D (Chen et al., 2008). From the previous results, it is concluded that *H. cordata* x10-1 could prove to be a useful source of terpenoid-free essential oil.

Terpenoids are plant defensive secondary metabolites. Besides the fragrance and biological activities, terpenoids are thought to act as essential ecological roles, providing defense against herbivorous insects or pathogens (Keeling and Bohlmann, 2006; Zulak and Bohlmann, 2010), attracting species-specific pollinators or the enemies of herbivores (Pichersky and Gershenzon, 2002), resisting oxidative and thermal stresses (Loreto et al., 1998), even serving as potential allelochemicals to communicate with other organisms or inhibit the germination and growth of neighbouring plants (Muller et al., 1968). However, being different from other *H. cordata* populations, the new line reported in the present study did not accumulate terpenes in the essential oil of the leaves. Therefore, the ecological role of the absence of terpenoids needs to be clarified in future studies. Terpenoids consist of one or more isoprene units, which are predominantly synthesized by the plastidial 2-

**Table 1.** Volatile oil composition of the leaves of *H. cordata* w01-100 and x10-1.

| <b>Compounds</b>           | <b>w01-100</b> | <b>x10-1</b> |
|----------------------------|----------------|--------------|
| $\alpha$ -Thujene          | 0.37           | -            |
| $\alpha$ -Pinene           | 2.17           | -            |
| $\beta$ -Phellandrene      | 15.96          | -            |
| $\beta$ -Pinene            | 2.46           | -            |
| $\beta$ -Myrcene           | 12.12          | -            |
| $\alpha$ -Terpinene        | 0.93           | -            |
| p-Cymene                   | 0.42           | -            |
| D-Limonene                 | 0.52           | -            |
| $\beta$ -Ocimene           | 3.91           | -            |
| $\gamma$ -Terpinene        | 2.52           | -            |
| Terpinolene                | 0.52           | -            |
| Nonanal                    | -              | 2.94         |
| 1-Nonanol                  | 0.65           | 0.11         |
| 4-Terpineol                | 4.02           | -            |
| Decanal                    | 15.82          | 42.64        |
| 1-Decanol                  | 0.59           | 5.85         |
| 2-Undecanone               | 1.80           | -            |
| Undecanal                  | 3.82           | 5.70         |
| 1-Undecanol                | 0.83           | -            |
| Cis-geraniol               | 0.94           | -            |
| Decanoic acid              | -              | 18.67        |
| Dodecanal                  | 21.15          | 15.04        |
| Caryophyllene              | 0.62           | -            |
| Undecanoic acid            | -              | 1.51         |
| 1-Dodecanol                | 0.65           | -            |
| 4-Tridecanone              | 1.51           | -            |
| 2-Tridecanone              | 0.93           | -            |
| $\gamma$ -Elemene          | 1.22           | -            |
| Tridecanal                 | 0.54           | 0.59         |
| Dodecanoic acid            | -              | 2.65         |
| Tetradecanal               | 1.27           | 2.09         |
| Hexadecanal                | 0.61           | 0.64         |
| Decanoic acid, decyl ester | -              | 0.37         |
| Terpenes                   | 48.69          | 0.00         |
| Monoterpenes               | 46.85          | 0.00         |
| Monoterpene hydrocarbons   | 41.90          | 0.00         |
| Monoterpene alcohols       | 4.95           | 0.00         |
| Sesquiterpenes             | 1.84           | 0.00         |
| Unbranched aldehydes       | 43.20          | 69.64        |
| Unbranched alcohols        | 2.72           | 5.96         |
| Unbranched ketones         | 4.24           | 0.00         |
| Unbranched acids           | 0.00           | 22.83        |
| Unbranched esters          | 0.00           | 0.37         |
| <b>Total</b>               | <b>98.85</b>   | <b>98.79</b> |

Note: Values mean the relative percentage amounts of the volatile oil components. (-) = not detected. Bold values represent terpenoid compounds or subclass of terpenoids.

C-methyl-D-erythritol 4-phosphate (MEP)/ mevalonate (MVA) pathway. Terpenoid synthases (TPS) are the

committed enzymes for terpenoid biosynthesis (Bick and Lange, 2003; Hemmerlin et al., 2003). TPS genes are

regulated by many factors such as plant hormones, signaling molecules and environmental stresses (Zhao et al., 2005). *H. cordata* usually produces terpenes, especially monoterpenes. However, *H. cordata* x10-1 showed the absence of terpenes. This suggests that the line may be a genetic mutation. The causes of variation are largely unknown. Study on gene level and regulation is needed to explore the phenomenon.

Our preliminary study showed that the essential oils of chemotype D and M exhibited differential antimicrobial activity against *Fusarium graminearum*, *Colletotrichum coccodes*, *Botrytis cinerea*, *Escherichia coli* and *Staphylococcus aureus*. This suggests that terpenes and aldehydes in the essential oil of *H. cordata* may represent different pharmacology activity. While no *H. cordata* accession is available to assay the respective pharmacology activity of terpenes and aldehydes until now. The new line reported in the present study may be a useful material to distinguish the role of terpenes and aldehydes of the essential oil of *H. cordata* in treating various symptoms.

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## REFERENCES

- Bick JA, Lange BM (2003). Metabolic cross talk between cytosolic and plastidial pathways of isoprenoid biosynthesis: unidirectional transport of intermediates across the chloroplast envelope membrane. *Arch. Biochem. Biophys.*, 415: 146-154.
- Chakraborti S, Sinha S, Sinha R (2006). High-frequency induction of multiple shoots and clonal propagation from rhizomatous nodal segments of *Houttuynia cordata* Thunb. - An ethnomedicinal herb of India. *In vitro Cell Dev. Plant*, 42: 394-398.
- Chen L, Wu W, Huang CY, Yang YX, Zheng YL (2008). Composition and variability of the essential oil of *Houttuynia* of China. *Chem. Nat. Compd.*, 44: 778-783.
- Han EH, Park JH, Kim JY, Jeong HG (2009). *Houttuynia cordata* water extract suppresses anaphylactic reaction and IgE-mediated allergic response by inhibiting multiple steps of FcεRI signaling in mast cells. *Food Chem. Toxicol.*, 47: 1659-1666.
- Hayashi K, Kamiya M, Hayashi T (1995). Virucidal effects of the steam distillate from *Houttuynia cordata* and its components on HSV-1, influenza virus, and HIV. *Planta Med.*, 61: 237-241.
- Hemmerlin A, Hoefler JF, Meyer O, Tritsch D, Kagan IA, Grosdemange-Billiard C, Rohmer M, Bach TJ (2003). Cross-talk between the cytosolic mevalonate and the plastidial methylerythritol phosphate pathways in tobacco bright yellow-2 cells. *J. Biol. Chem.*, 278: 26666-26676.
- Keeling CI, Bohlmann J (2006). Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytol.*, 170: 657-675.
- Kim SK, Ryu S, No J, Choi S, Kim Y (2001). Cytotoxic alkaloids from *Houttuynia cordata*. *Arch. Pharm. Res.*, 24: 518-521.
- Lau KM, Lee KM, Koon CM, Cheung CSF, Lau CP, Ho HM, Lee MYH, Au SWN, Cheng CHK, Lau CBS, Tsui SKW, Wan DCC, Waye MMY, Wong KB, Wong CK, Lam CWK, Leung PC, Fung KP (2008). Immunomodulatory and anti-SARS activities of *Houttuynia cordata*. *J. Ethnopharmacol.*, 118: 79-85.
- Liang HX (1995). On the evolution and distribution in Saururaceae. *Acta Bot. Yunnanica*, 17: 255-267.
- Liang M, Qi M, Zhang C, Zhou S, Fu R, Huang J (2005). Gas chromatography-mass spectrometry analysis of volatile compounds from *Houttuynia cordata* Thunb. after extraction by solid-phase microextraction, flash evaporation and steam distillation. *Anal. Chim. Acta*, 531: 97-104.
- Loreto F, Förster A, Dürr M, Csiky O, Seufert G (1998). On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. *Plant Cell Environ.*, 21: 101-107.
- Muller WH, Lorber P, Haley B (1968). Volatile growth inhibitors produced by *Salvia leucophylla*: effect on seedling growth and respiration. *Bull. Torrey Bot. Club*, 95: 415-422.
- Nuengchamnong N, Krittasilp K, Ingkaninan K (2009). Rapid screening and identification of antioxidants in aqueous extracts of *Houttuynia cordata* using LC-ESI-MS coupled with DPPH assay. *Food Chem.*, 117: 750-756.
- Ogle B, Tuyet H, Duyet H, Xuan Dung N (2003). Food, feed or medicine: the multiple functions of edible wild plants in Vietnam. *Econ. Bot.*, 57: 103-117.
- Pichersky E, Gershenzon J (2002). The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Curr. Opin. Plant Biol.*, 5: 237-243.
- Toda S (2005). Antioxidative effects of polyphenols in leaves of *Houttuynia cordata* on protein fragmentation by copper-hydrogen peroxide *in vitro*. *J. Med. Food*, 8: 266-268.
- Wu W, Zheng YL, Chen L, Wei YM, Yan ZH (2005a). Genetic diversity among the germplasm resources of the genus *Houttuynia* Thunb. in China based on RAMP markers. *Genet. Resour. Crop. Evol.*, 52: 473-482.
- Wu W, Zheng YL, Chen L, Wei YM, Yan ZH, Yang RW (2005b). PCR-RFLP analysis of cpDNA and mtDNA in the genus *Houttuynia* in some areas of China. *Hereditas*, 142: 24-32.
- Wu W, Zheng YL, Chen L, Wei YM, Yang RW, Yan ZH (2005c). Evaluation of genetic relationships in the genus *Houttuynia* Thunb. in China based on RAPD and ISSR markers. *Biochem. Syst. Ecol.*, 33: 1141-1157.
- Wu W, Zheng YL, Yang RW, Chen L, Wei YM (2003). Variation of chromosome number and cytotoxicity of *Houttuynia cordata* Thunb. from China. *Acta Phytotaxon Sin.*, 41: 245-257.
- Xu YW, Zou YT, Husaini AM, Zeng JW, Guan LL, Liu Q, Wu W (2011). Optimization of potassium for proper growth and physiological response of *Houttuynia cordata* Thunb. *Environ. Exp. Bot.*, 71: 292-297.
- Zhao J, Davis LC, Verpoorte R (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.*, 23: 283-333.
- Zulak KG, Bohlmann J (2010). Terpenoid biosynthesis and specialized vascular cells of conifer defense. *J. Integr. Plant Biol.*, 52: 86-97.