Distribution and Composition Analysis of Essential Oils Extracted from Different Parts of *Cupressus funebris* and *Juniperus chinensis*

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The quantitative comparison of essential oils extracted from various parts of *Cupressus funebris* and *Juniperus chinensis* was studied. With increased height, the content of essential oils from branches decreased, while the content of essential oils from leaves increased for both species. A maximum amount of essential oils were found in the west and a minimum in the east of branches and leaves for the two species. Moreover, the content of essential oils in trees with a knot was higher than in those without a knot. The order of essential oil content was as follows: leaf > fine root > coarse root > bark > bough > branch > trunk in *C. funebris*, and leaf > fine root > bark > coarse root > bough > trunk > branch in *J. chinensis*. The essential oils extracted from various parts of *C. funebris* and *J. chinensis* were analyzed by gas chromatography-mass spectrometry. A total of 67, 33, 69, 65, and 69 components were identified from the roots, trunks, barks, branches, and leaves of *C. funebris*, respectively. A total of 72, 46, 79, 55, and 82 components were identified from the roots, trunk, bark, branches, and leaves of *J. chinensis*, respectively.

Keywords: Essential oils; Cupressus funebris; Juniperus chinensis; Chemical composition; Distribution

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INTRODUCTION

The genus *Cupressus*, which belongs to the family Cupressaceae, originated in the Mediterranean region and includes approximately 17 species distributed in the southern part of North America, East Asia, and Mediterranean region. In China, there are nine species and one variety, out of which four species are endemic to China. *Cupressus funebris* is one of these four endemic species (Fu *et al*. 1999). *Juniperus chinensis* is an ornamental plant that also belongs to the family Cupressaceae (Hora 1981), which has more than 100 cultivars.

Most of the research literature focuses on forest resource management and biodiversity, while only a few of them discuss the essential oils of *C*. *funebris* and *J. chinensis* (Zheng *et al*. 2011; Hou *et al*. 2013; Li *et al*. 2015). The current research on essential oils extracted from C*upressus funebris* and *Juniperus chinensis* includes some detailed reports of the chemical constituents of these essential oils. Pierre-Leandri *et al*. (2003) and Duquesnoy *et al*. (2006) found 59 and 46 compounds, respectively, in the oils of C. *funebris*, while Pu and Huang (1999) and Raina *et al*. (2005) found and identified 34 and 33 compounds, respectively, in the oils of *J. chinensis*. However, most of these studies focused on studying the biological pest control and biological and antimicrobial activities of the major constituents of essential oils from *C. funebris* and *J. chinensis* (Dolan *et al*. 2007; Lee *et al*. 2009; Carroll *et al*. 2011; Giatropoulos *et al*. 2013). Only a few studies have been conducted in terms of the distribution of essential oils of *C. funebris* and *J. chinensis*. In other species like *Pinus roxburghii*, *Laurelia sempervirens, Drimys winteri*, *Cinnamomum osmophloeum*, and *Litsea cubeba*, research on the essential oils from different parts of the plants has been conducted (Cheng *et al*. 2006; Wang and Liu 2010; Zapata and Smagghe 2010; Salem *et al*. 2014).

The aim of this paper was: (a) to investigate the distribution of the essential oils from different parts of *C. funebris* and *J. chinensis*; and (b) to assess the chemical composition of the obtained essential oils from the different parts of *C. funebris* and *J. chinensis*.

EXPERIMENTAL

Materials

Cupressus funebris was collected from the Shiyang town of Dujiangyan, in the Sichuan province, China. The tree was 13 years old. *Juniperus chinensis* was collected from the Hesheng garden of Dujiangyan, Sichuan, China, and the height of the tree was taller than 3 m. After the logs were cut down, the branches and leaves of different heights and different directions were collected and returned to the laboratory for pulverization after air-drying.

The experimental material was grouped according to diameter of branches and roots, as follows: one-year branches and leaves, bough (diameter $\geq$ 2.0 mm), fine root (diameter $\leq$ 2.0 mm), and coarse root (diameter $\geq$ 2.0 mm). The crown of the tree was divided into trisections along the high direction, and the branches and leaves were harvested in each height interval.

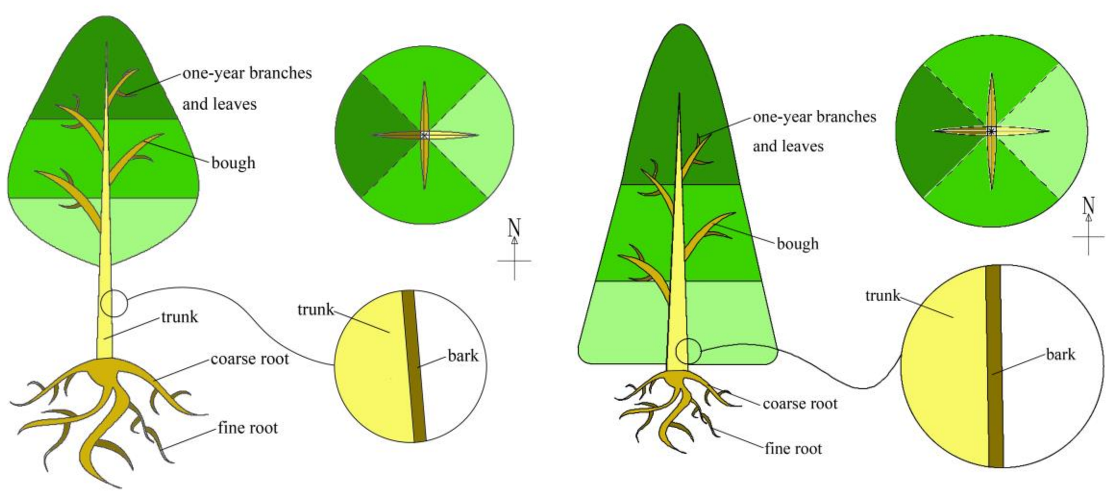

Fig. 1. The schematic diagram of sampling and positions in *Cupressus funebris* (left) and *Juniperus chinensis* (right)

The experimental material was grouped according to tree direction. The direction of the tree was marked before logging, and the branches and leaves were packed separately, according to different direction. It was ensured that there was a uniform distribution in height when sampling.

The experimental material also was grouped according to inner and outer sides of xylem. After removing the bark from the wood, a straight line was drawn from the heart-center to divide it into three equal divisions. The middle division was considered the inside of xylem, and both the ends were considered the outside of xylem. The xylem parts were sawed after aforementioned internal and external classification into a wood specimen of approximately 2 cm × 2 cm × 10 cm. The wood specimen was classified as wood specimen with knot if any knot was seen, otherwise it was classified as wood specimen without knot. The bark was peeled off from the xylem, and if the cut off portion was approximately 5 cm × 5 cm in size near the branch or knot, then it was classified as bark specimen with knot, otherwise, it was classified as bark specimen without knot.

After the detailed classification and pretreatment was completed as mentioned above, the materials were crushed for testing after air-drying.

Methods

Distribution analysis experiment

Essential oils from the roots, trunk, bark, branches, and leaves of *C. funebris* and *J. chinensis* were extracted *via* a steam-distillation method, the set-up of the equipment includes round bottom flask, heating device, condenser, and essential oil collector.

In the *Cupressus funebris* experiment, 600 mL of NaCl aqueous solution with mass fraction of 2% was added to the 100 g powdered standard specimens, which was heated by thermostatically controlled heating device. In the *Juniperus chinensis* experiment, 600 mL of NaCl aqueous solution with mass fraction of 1% was added to the 100 g powdered standard specimens, which was heated by thermostatically controlled heating device. The distillation was continued until no distillate was seen, and 3 replicates were set to observe the extent of oil extraction (Dai *et al*. 2011; Wu *et al*. 2015).

Light yellow oil was obtained after drying of the essential oils for 15 min with sodium sulfate anhydrous (Na_2SO_4). The dried essential oils were diluted to a 0.04 g/mL solution with hexane (chromatographic purity).

Composition analysis experiment

The essential oils were analyzed by capillary gas chromatograph equipped with flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS) using an Agilent GCMS7890B-5977 system equipped with a FID detector (Agilent Technologies, Palo Alto, CA, USA). Gas chromatographic separation with a DB-17MS capillary column (30 m × 0.25 mm, i.d. 0.25-μm film) was used with helium as the carrier gas (1.2 mL/min). The GC oven temperature was programmed at 80 °C (held 4 min), raised to 135 °C (held 5 min) at 10 °C /min, and subsequently to 235 °C (held 2 min) at 5 °C /min. The injector temperature was set at 270 °C, the GC split ratio was adjusted to 10:1, and the sample volume was 1.0 μL. The MS conditions were as follows: ionization voltage of 70 eV, mass range 15 μ to 500 μ, and ion source temperature at 230 °C.

RESULTS AND DISCUSSION

Essential Oil Distribution Analysis of *C. funebris* and *J. chinensis*

Differences in essential oil content from different parts

Standard analysis of variance (ANOVA) methods were applied to essential oil distribution analysis of *C. funebris* and *J. chinensis* by using IBM SPSS Statistics (Version 19, IBM, Armonk, NY, USA).

Table 1. Analysis of Variance of Extraction Extent of Essential Oil from Different Parts

	Sources	Sum of Squares	df	Mean Square	F	p
Cupressus funebris	Between groups	65.670	6	10.945	1532.289	<0.0001**
	Within groups	0.100	14	0.007		
	Total	65.770	20			
Juniperus chinensis	Between groups	96.483	6	16.080	912.676	<0.0001**
	Within groups	0.247	14	0.018		
	Total	96.730	20			

Note: * indicates significant difference at 0.05 level, and ** indicates significant difference at 0.01 level.

Table 1 shows the significant differences among the extraction extent of essential oils from different parts, both in *C. funebris* and *J. chinensis* ($p < 0.01$). Most of the essential oil content comes from the leaves and the least amount comes from the trunk in *Cupressus funebris.* Most of the essential oil content comes from the leaves and the least amount comes from the branches in *Juniperus chinensis*. Experimental results indicated that the order of essential oil content was: leaf > fine root > coarse root > bark > bough > branch > trunk in *Cupressus funebris*, and leaf > fine root > bark > coarse root > bough > trunk > branch in *Juniperus chinensis*, as shown in Fig. 2.

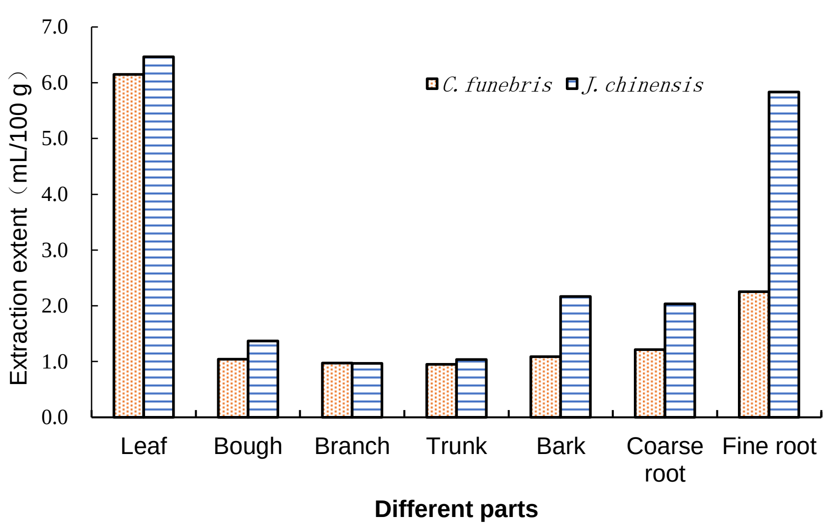

Fig. 2. The extraction extent of different parts in *Cupressus funebris* and *Juniperus chinensis*

The reason for the variation in essential oil content from the different parts could have been that the secondary metabolites of trees were released from the leaves into the environment above the ground and from roots under the ground, which resulted in a higher amount of essential oils in these two parts. The similar distribution of essential oils in different parts of *Cupressus funebris* and *Juniperus chinensis* may have been because the composition, distribution, and content of secondary metabolites was influenced by the phylogeny of plants.

Influence of trunk height on essential oil content

Table 2 shows that there were significant differences among the essential oil extraction extent of xylem extracted from the parts taken from different heights of *Cupressus funebris* ($p < 0.01$), both in inner and outer parts of xylem, with more significance in inner than in outer parts of xylem.

Table 2. Analysis of Variance of Essential Oil Extraction Extent of Xylem Taken from Different Part Heights of *Cupressus funebris*

	Sources	Sum of Squares	df	Mean Square	F	p
Inside Xylem	Between groups	1.416	2	0.708	70.778	<0.0001**
	Within groups	0.060	6	0.010		
	Total	1.476	8			
Outside Xylem	Between groups	0.136	2	0.068	30.500	0.001 **
	Within groups	0.013	6	0.002		
	Total	0.149	8			

Note: * indicates significant difference at 0.05 level, and ** indicates significant difference at 0.01 level.

As shown in Fig. 3, the essential oil content of the inner parts of xylem decreased as the height increased, while an opposite trend was observed for the essential oil content of the outer parts of xylem. Therefore, the extraction extent of inner parts of xylem was greater than the outer ones.

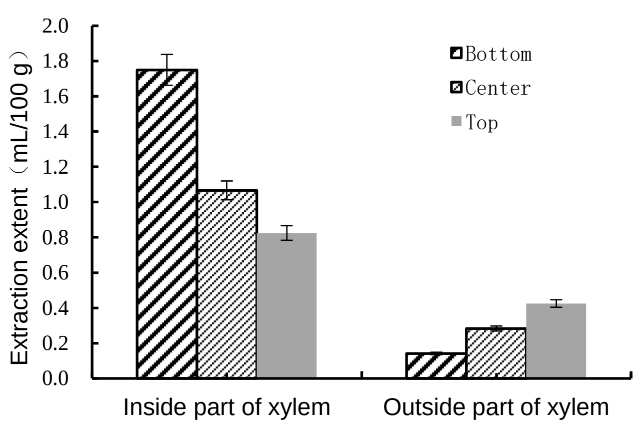

Fig. 3. Essential oil extraction extent in xylem extracted from parts taken from different heights in *Cupressus funebris*

Table 3. Essential Oil Extraction Extent in Xylem Extracted from Parts Taken from Different Heights of *Juniperus chinensis*

	Sources	Sum of Squares	df	Mean Square	F	p
Inner Xylem	Between groups	2.407	1	2.407	103.143	0.001 **
	Within groups	0.093	4	0.023		
	Total	2.500	5			
Outer Xylem	Between groups	1.707	1	1.707	256.000	<0.0001**
	Within groups	0.027	4	0.007		
	Total	1.733	5			

Note: * indicates significant difference at 0.05 level, and ** indicates significant difference at 0.01 level.

As the trunk diameter of *Juniperus chinensis* is small and the height is generally low, the height was only divided into the bottom and top parts. Table 3 shows that there were significant differences among the essential oil extraction extents of xylem taken from different heights of *Juniperus chinensis* ($p < 0.01$) in both the inner and outer parts of xylem, with more significance in outer than inner parts of xylem. As shown in Fig. 4, the essential oil content of both the inner and outer parts of xylem decreased as height increased. Therefore, the extraction extent of inner parts of xylem was greater than the outer ones.

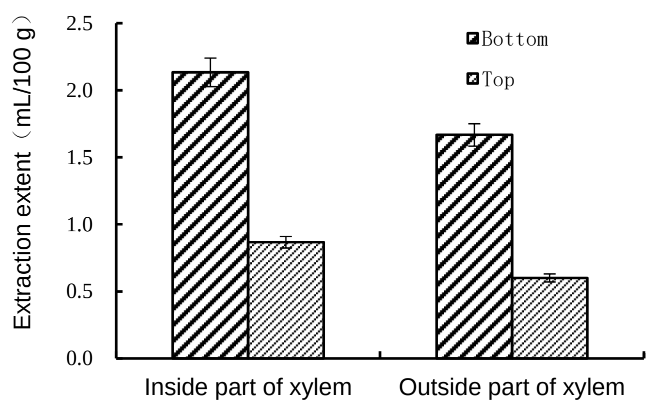

Fig. 4. Essential oil extraction extent of xylem taken from different heights of *J. chinensis*

The essential oil extraction extent from the inner and outer parts of *Cupressus funebris* xylem showed different trends with respect to an increase in trunk height, while the opposite conclusion was drawn for *Juniperus chinensis.*

There was a significant difference between the inner and outer parts of xylem due to the larger xylem diameter of *C. funebris*. However, there was no significant difference between the inner and outer parts of xylem due to the smaller diameter of xylem of *J. chinensis,* which led the authors to conclude that the essential oil content observed was consistent in the two species.

Influence of branch and leaf height on essential oil content

Table 4 shows that there was a significant difference in the essential oil extraction extent from the branches and leaves harvested from different heights of *Cupressus funebris* ($p < 0.01$). The branch essential oil extraction extent gradually decreased as height increased. In contrast, the essential oil extraction extent of leaves gradually increased as height increased (Fig. 5).

Table 4. Analysis of Variance of Essential Oil Extraction Extent of Branch and Leaf Taken from Different Heights of *Cupressus funebris*

	Sources	Sum of Squares	df	Mean Square	F	p
Branch	Between groups	1.056	2	0.528	67.857	<0.0001**
	Within groups	0.047	6	0.008		
	Total	1.102	8			
Leaf	Between groups	12.987	2	6.493	254.087	<0.0001**
	Within groups	0.153	6	0.026		
	Total	13.140	8			

Note: * indicates significant difference at 0.05 level, and ** indicates significant difference at 0.01 level.

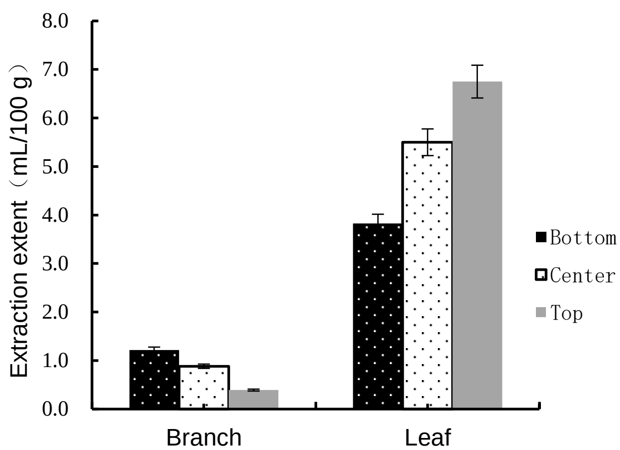

Fig. 5. Essential oil extraction extent of branch and leaf taken from different heights of *C. funebris*

Table 5. Analysis of Variance of Essential Oil Extraction Extent of Branch and Leaf Taken from Different Heights of *Juniperus chinensis*

	Sources	Sum of Squares	df	Mean Square	F	p
Branch	Between groups	0.287	2	0.143	16.125	0.004 **
	Within groups	0.053	6	0.009		
	Total	0.340	8			
Leaf	Between groups	66.107	2	33.053	1025.793	<0.0001**
	Within groups	0.193	6	0.032		
	Total	66.300	8			

Note: * indicates significant difference at 0.05 level, and ** indicates significant difference at 0.01 level.

Table 5 shows that there was a significant difference in the essential oil extraction extent of branches and leaves taken from different heights of *Juniperus chinensis* ($p < 0.01$), with the leaf group showing a more significant difference than the branch group. The essential oil extraction extent of branches gradually, but slowly, decreased with height. In contrast, the essential oil extraction extent of leaves gradually, but sharply, increased with height (Fig. 6).

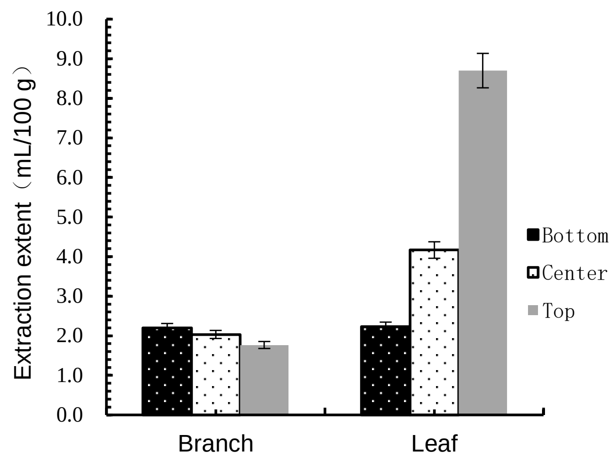

Fig. 6. Essential oil extraction extent of branch and leaf from different height parts in *Juniperus chinensis*

The increase of leaf essential oil extraction extent could have been due to light intensity and illumination time. The increase in light intensity could have promoted the accumulation of secondary metabolites of *Cupressus funebris* and *Juniperus chinensi*s. A greater light intensity resulted in a greater accumulation of secondary metabolites, which may have led to the increased content of extracted essential oils.

The longer exposure time of light irradiation might have strongly increased the content of secondary metabolites, thus leading to an increase in essential oil content of leaves from the bottom to top. However, the decrease of the essential oil content of branches with height could have been due to the transport and accumulation of secondary metabolites in leaves, which increased the consumption of secondary metabolites in branches, reducing the essential oil content of the branches.

Influence of branch and leaf direction on essential oil content

Table 6 shows that there was a significant difference in the essential oil extraction extent from branches and leaves taken from different directions of *C. funebris* ($p < 0.01$), with the branch group showing more significant differences than the leaf group. Figure 7 shows that the maximum values occurred in the western direction, and the minimum values occurred in the eastern direction, for both branches and leaves. There were a few differences between the south and north directions. The essential oil content of leaves was much higher than that of branches.

Table 6. Analysis of Variance of Essential Oil Extraction Extent of Branch and Leaf from Different Directions of *Cupressus funebris*

	Sources	Sum of Squares	df	Mean Square	F	p
Branch	Between groups	0.560	3	0.187	32.000	<0.0001**
	Within groups	0.047	8	0.006		
	Total	0.607	11			
Leaf	Between groups	0.453	3	0.151	13.949	0.002 **
	Within groups	0.087	8	0.011		
	Total	0.540	11			

Note: * indicates significant difference at 0.05 level, and ** indicates significant difference at 0.01 level.

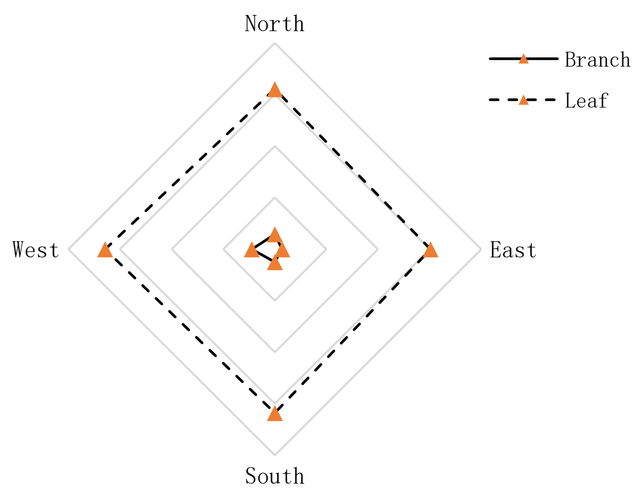

Fig. 7. Essential oil extraction extent of branch and leaf from different directions of *C. funebris*

Table 7. Analysis of Variance of Essential Oil Extraction Extent of Branch and Leaf from Different Directions of *Juniperus chinensis*

	Sources	Sum of Squares	df	Mean Square	F	p
Branch	Between groups	0.007	3	0.002	0.533	0.672
	Within groups	0.033	8	0.004		
	Total	0.040	11			
Leaf	Between groups	3.269	3	1.090	93.405	<0.0001**
	Within groups	0.093	8	0.012		
	Total	3.363	11			

Note: * indicates significant difference at 0.05 level, and ** indicates significant difference at 0.01 level.

Table 7 shows that there was a significant difference in the essential oil extraction extent from leaves taken from different directions of *Juniperus chinensis* ($p < 0.01$), but no significant differences were observed in the essential oil extraction extent of branches from different directions of *Juniperus chinensis*. As shown in Fig. 8, the order of essential oil extraction extent of leaves was: West > North > South > East. There was no significant difference in the result of essential oil extraction extent of branches. In addition, the essential oil of leaves was greater than that of branches, but the difference was small.

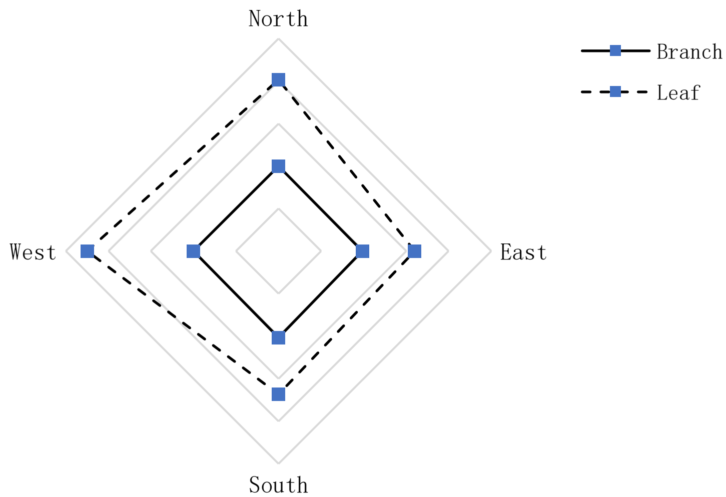

Fig. 8. Essential oil extraction extent of branch and leaf from different directions of *J. chinensis*

The direction had a significant influence on the extraction extent of essential oils from the leaves of *Cupressus funebris* and *Juniperus chinensis*. The reason may have been related to the intensity and duration of illumination, because the leaves and branches of the west direction were affected by the high intensity and duration of illumination, which increased the secondary metabolites content, subsequently, leading to a high essential oil content.

Influence of knot on essential oil content of Cupressus funebris

Table 8 shows that there was a significant difference in the essential oil extraction extents of xylem and bark with or without knot of *C. funebris* ($p < 0.01$), and the bark group showed a more significant difference than the xylem group. Figure 9 shows that the essential oil extraction extent with and without knot gradually increased as height increased, which was consistent with the previously mentioned results in Fig. 3 about the outer parts of xylem; the reason was because the knot was located in outer parts of xylem. As shown in Fig. 10, the essential oil content with knot was greater than that without the knot for both the bark and xylem parts in *C. funebris*.

Table 8. Analysis of Variance of Essential Oil Extraction Extent of Knot of *Cupressus funebris*

	Sources	Sum of Squares	df	Mean Square	F	p
Xylem	Between groups	0.667	1	0.667	50.000	0.002 **
	Within groups	0.053	4	0.013		
	Total	0.720	5			
Bark	Between groups	1.307	1	1.307	784.000	<0.0001**
	Within groups	0.007	4	0.002		
	Total	1.313	5			

Note: * indicates significant difference at 0.05 level, and ** indicates significant difference at 0.01 level.

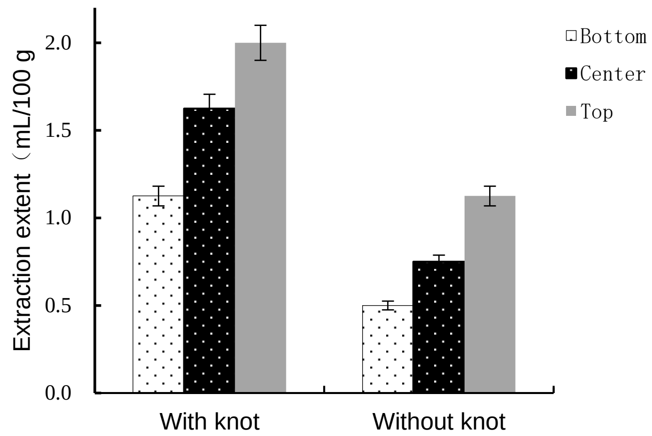

Fig. 9. Essential oil extraction extent of xylem with and without knot of *Cupressus funebris*

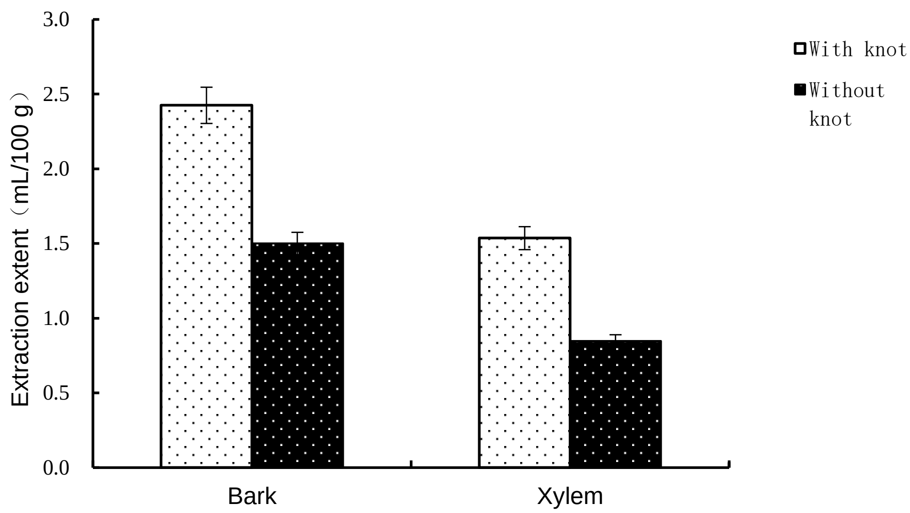

Fig. 10. Essential oil extraction extent of bark and xylem with and without knot of *Cupressus funebris*

Composition Analysis in *Cupressus funebris* and *Juniperus chinensis*

Quantification was performed using percentage peak area calculations with the GC–FID. The chromatographic peaks were preliminarily identified by means of an automatic search of the NIST library of mass spectra. The obtained mass spectra were then compared with those reported in previous literature (Adams 2007). The chemical compounds were analyzed qualitatively using the relative reservation index and analyzed quantitatively by peak area normalization measurements.

Composition analysis in Cupressus funebris

A total of 67, 33, 69, 65, and 69 volatile components were identified from the roots, trunk, bark, branches, and leaves, which accounted for 82.8%, 87.8%, 73.2%, 60.7 %, and 95.6% of the total essential oils, respectively.

The terpenoid content was high in essential oil from all five parts of *Cupressus funebris*. Thujopsene, ferruginol, and cedrol were found in all five parts. The content of ferruginol and cedrol was highest in the trunk, while the content of thujopsene was highest in bark. The content of longifolene was highest at 14.5% in roots, while the content of β-phellandrene was highest at 11.0% in leaves. Cedrol was the most abundant compound in the trunk, bark, and branches, with relative contents at 38.0%, 9.7%, and 14.6%, respectively.

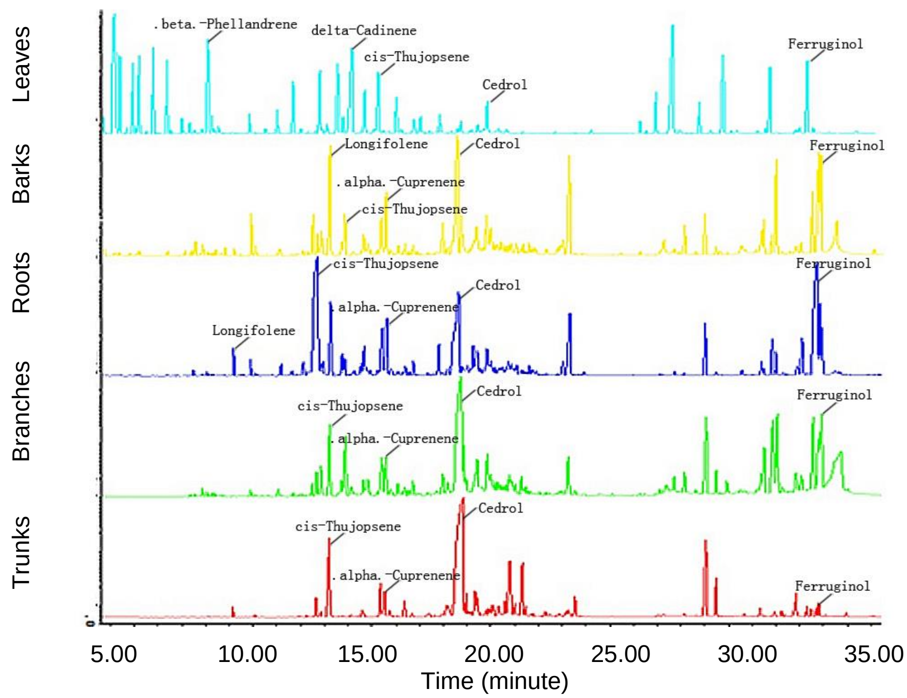

Fig. 11. Ion chromatogram of essential oil extracted from leaves, barks, roots, branches, and trunks of *Cupressus funebris*

Composition analysis in Juniperus chinensis

A total of 72, 46, 79, 55, and 82 volatile components were identified from the roots, trunk, bark, branches, and leaves, which accounted for 87.5%, 78.3%, 82.5%, 69.3 %, and 88.0% of the total essential oils, respectively.

The terpenoid content was high in essential oil from all five parts of *Juniperus chinensis*, just like *Cupressus funebris*. Thujopsene, α-Cuparene, α-cuprenene, Cedrol, Ferruginol, and Cedren-13-ol,8-, were found in all five parts. Thujopsene, α-cuparene, and α-cuprenene were the highest in leaves, with relative contents at 15.29%, 2.97%, and 2.55%, respectively.

The content of cedrol and ferruginol was highest in trunks, while the content of cedren-13-ol,8- was highest in branches. Cedrol was the most abundant compound in roots, trunk, bark, and branches, with relative contents at 18.1%, 34.0%, 9.0%, and 9.4%, respectively.

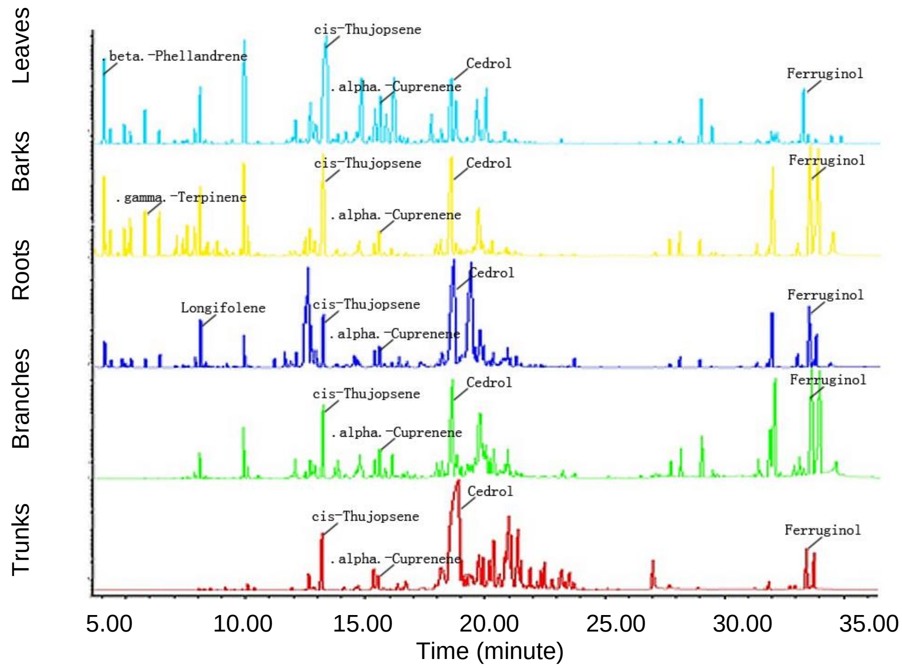

Fig. 12. Ion chromatogram of essential oil extracted from leaves, barks, roots, branches, and trunks of *Juniperus chinensis*

CONCLUSIONS

1. The essential oil content decreased with the increase in height of branches for both *Cupressus funebris* and *Juniperus chinensis,* but the essential oil content increased with the increase in height of leaves for both the species.
2. The essential oil content of the inner parts of xylem decreased with increased height, while an opposite trend was observed for that of the outer parts of xylem in *Cupressus funebris*. However, the essential oil content of both the inner and outer parts of xylem decreased increased height in *Juniperus chinensis*.
3. Maximum values of essential oil content occurred in the western direction, and the minimum values occurred in the eastern direction for leaves of both *C. funebris* and *J. chinensis*. Maximum values of essential oil content occurred in the western direction, and the minimum values occurred in the eastern direction only for branch of *C. funebris*, but there was no significant difference for branch of *J. chinensis*.
4. The essential oil content with knot was greater than that without knot for both the bark and xylem parts in *C. funebris* and *J. chinensis*. The order of essential oil content was: leaf > fine root > coarse root > bark > bough > branch > trunk in *C. funebris*, and leaf > fine root > bark > coarse root > bough > trunk > branch in *J. chinensis.*
5. Thujopsene, ferruginol, and cedrol were found in all five parts of *C. funebris*. The content of longifolene was highest in roots and the content of β-phellandrene was highest in leaves. The cedrol was the most abundant compound in the trunks, barks, and branches of *C. funebris*. Moreover, thujopsene, α-cuparene, α-cuprenene, cedrol, ferruginol, and cedren-13-ol,8- were found in all five parts of *J. chinensis*. The

thujopsene content was highest in leaves, while cedrol was the most abundant compound in roots, trunks, barks, and branches of *C. funebris*.

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