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Variation of Concentration of Terpenes in *Chrysanthemum boreale*

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산국에서의 Terpenes 함량의 변이

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ABSTRACT

The monoterpenes and sesquiterpenes are analysed in the leaf and stem of *Chrysanthemum boreale* using gas chromatography-mass spectrometry (GC-MS). The total amount of sesquiterpenes are always higher than monoterpenes in both leaf (2.0~3.4 times) and stem (1.6~8.3 times). The mono- and sesquiterpenes yields of the leaf are higher than the stem. There was no significant difference among the leaf developmental stages, while those of stem were varied. Seventeen monoterpenes and 9 sesquiterpenes compound in this plants comprised more than 5% of the mean total monoterpenes and the total sesquiterpenes in each dates. Among leaf monoterpenes, the concentration of (+)-Limonene and unknown compound no. 13 (Retention time, R.T.=17.28) varied significantly during leaf growing season, and the concentration of unknown compound no. 7 (R.T. =35.04) and no. 9 (R.T.=35.71) varied in the leaf sesquiterpenes. Similarly the results from the leaf, the concentration of five monoterpenes in stem also varied significantly during maturing period, and much varied in seven compounds of stem sesquiterpene. The major sesquiterpenes of leaf and stem were α-Humulene and compound no. 2 (R.T.=26.19).

Key words: α-Humulene, *Chrysanthemum boreale*, Monoterpenes, Sesquiterpenes, (+)-Limonene.

INTRODUCTION

Terpenes are synthesized from acetyl CoA via the mevalonic acid pathway. All terpenes are derived from the union of 5-carbon elements that have the branched carbon skeleton of isoprene. Monoterpenes (C_{10}), sesquiterpenes (C_{15}), and diterpenes (C_{20}) are produced by the sequential addition of C_5 units. Triterpenes (C_{30}) are formed from two C_{15} units and tetraterpenes (C_{40}) from two C_{20} units. Among them, mixtures of volatile monoterpenes and sesquiterpenes, called essential oils, which lend a characteristic odour to plant foliage. Plant essential oils have been widely used in taxonomic (Williams *et al.* 1995), phylogenic (Harbone and Turner 1984), microbial (White 1986) and ecological (Langenheim 1994, Kim and Langenheim 1994) studies. Essential oils have wellknown insect repellent properties (Edwards *et al.* 1993). Especially many monoterpenes and their derivates are important agents of insect toxicity (Mattson *et al.* 1988, Croteau *et al.* 1981) and allelochemicals (Kil *et al.* 1994, Jimenez-Osornio *et al.* 1996). Many ecologists (Langenheim *et al.* 1986, Picman 1986, Goralka *et al.* 1996, Li *et al.* 1995) have suggested that plants with these compounds have a broad range of ecological distribution through evolution, terpene compounds may serve as defense mechanisms against herbivores, fungi, bacterial pathogens and other plants.

There are much amounts of monoterpenes and their derivates in the genus *Chrysanthemum*. The monoterpene esters called pyrethroids occurred in the leaves and flowers of *Chrysanthemum* species (Taiz 1991). Both natural and synthetic pyrethroids are popular commercial insecticides because of their low persistence in the environment and their negligible toxicity to mammals. *Chrysanthemum* species have a lot of sesquiterpenes, which are known to be antiherbivorous, antifungal, and antibacterial pathogens. They also taste bitter to humans (Rafii *et al.* 1992, 1996). *Chrysanthemum* species is an perennial herb that has been used for traditional purgative for intestinal bacteria, for headache and dizziness medicine and many other medicinal purposes in Korea. Moreover *C. boreale* is widespread species in Korea. However the ecological concentration of terpenes in *C. boreale* have not yet been tested. Therefore in order to serve a basic information about the biological activity of terpenes from *C. boreale*, concentrational variation of monoterpenes and sesquiterpenes were studied during growing season in the leaf and stem of *C. boreale*. Since this study was intended out to find the difference between the total amounts of monoterpenes and sesquiterpenes, the compounds were not identified in detail.

MATERIALS AND METHODS

Chrysanthemum boreale was collected from different five sites at Mt. Muhak, sealed in plastic bags, and transported to the laboratory during maturing period approximately one week intervals. Plants were separated into leaf and stem, and immediately three grams of subsamples were ground with pure sand and extracted with n-pentane (approximately 50ml) and one ml internal standard (1% tetradecane). Plant extracts were filtered with sodium sulfate and concentrated under a gentle stream of nitrogen gas. One μl of each extract was injected to injector of the combined GC-MS (Gas Chromatograpy-Mass Spectrophotometry : HP-5890). GC fitted with a 30 m HP-5MS capillary column with an inside diameter of 0.25mm and a flame ionization detector. The injector temperature, dectector temperature, and flow rate were 220℃, 320℃, and 1.8ml/min, respectively. The initial oven temperature was 37℃ for five minutes, and increased to 180℃ at a rate of 5℃ per minute, then by 20℃ per minute until 320℃ (Kim and Langenheim 1994).

Monoterpenes and sesquiterpenes were identified by the use of known standards (Aldrich Chem. Co.) of individual compounds and quantified by the use of tetradecane as an internal standard. The ANOVA for variation in oil components was calculated following Sokal and Rohlf (1973) and computed using the Excell program (ver. 4.0). T-testing was performed for the difference of terpenes in the leaf and stem.

RESULTS AND DISCUSSION

Gas chromatographic assessment of the extraction from leaf and stem of *C. boreale*. Approximately 17 monoterpenes (Fig. 1), and 9 sesquiterpenes (Fig. 2) were dectected in *C. boreale*, however many were present only in small or trace amounts. Because of the complexity of chromatograms, a limited number of unidentified peaks were examined. In this study, although monoterpenes and sesquiterpenes do not enough to identify, yield absolute concentrations, relative differences and an assessment of seasonal variation are valid.

The total concentrations of sesquiterpenes were higher (from 2.0 times to 3.4 times) than monoterpenes in leaf of *C. boreale* (Fig. 3). The total concentration of monoterpenes in the leaf of *C. boreale* ranged

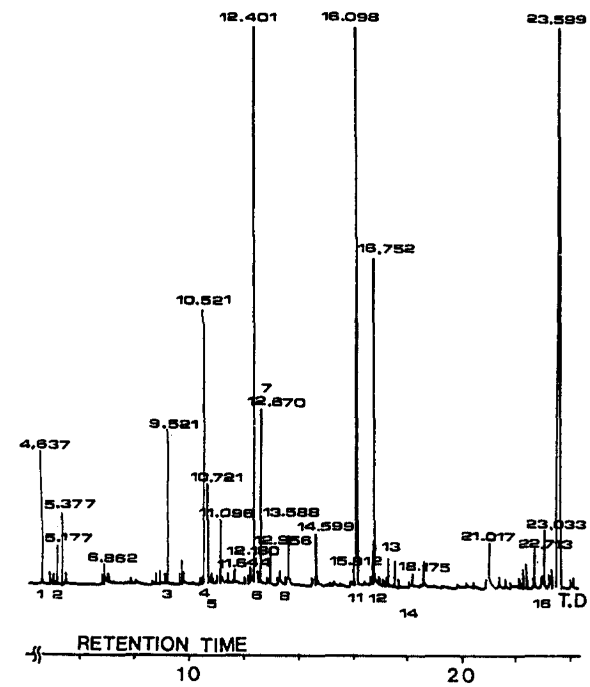

Fig. 1. Gas chromatographic assessment of the extraction from *C. boreale* leaf monterpenes in May 30. Many monoterpenes are present only in small or trace amounts. Number means monoterpenes compounds with the same No. in Table 1. Compounds represented after T.D. are sesquiterpenes.

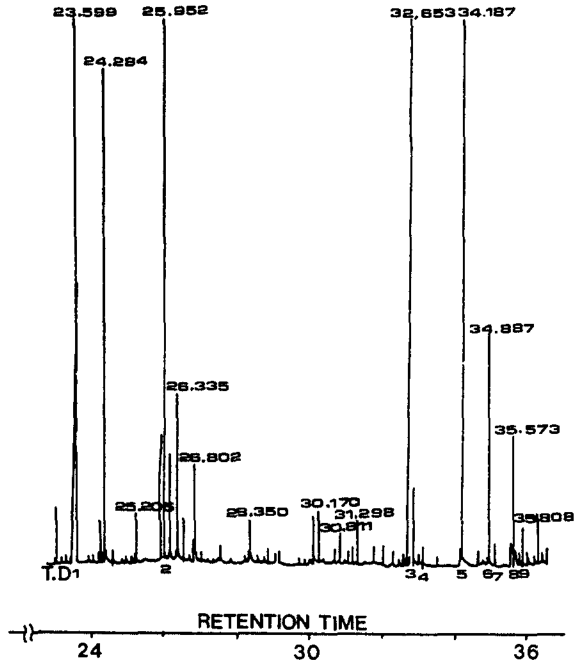

Fig. 2. Gas chromatographic assessment of the extraction from *C. boreale* leaf sesquiterpenes in May 30. Many sesquiterpenes are present only in small of trace amounts. Number means sesquiterpenes compounds with the same No. in Table 2. Compounds represented before T.D. are monoterpenes.

from 0.062 (mg/g f.w.) to 0.116 (mg/g f.w.), there is no significant difference (F=1.863, p=0.1585) among the developmental stages. And also there is no significant difference (F=2.30, p=0.096) in the total concentration (0.204 mg/g f.w. to 0.302 mg/g f.w.) of leaf sesquiterpenes in developmental stages. Fig. 4 shows that the total amount of monoterpenes and sesquiterpenes in the stem of *C. boreale*. There were apparently significant differences in mono- and sesquiterpenes concentrations. The amounts of monoterpenes in the stem of *C. boreale* were high ranging from 0.011 to 0.034 (mg/g f.w.), and that of sesquiterpenes were from 0.055 to 0.173 (mg/g f.w.) for all dates. The amounts of sesquiterpenes were, same trend the results of leaf, significantly higher (1.6~8.3 times) than monoterpenes for all dates. Turning to the leaf results, there are significant differences in the total amounts of stem mono-(F=4.

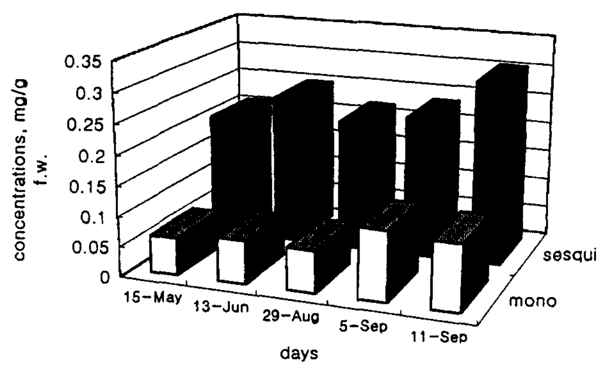

Fig. 3. The variation of the total concentrations between monoterpenes and sesquiterpenes in the leaf of *Chrysanthemun boreale*. Means in each column come fron five measurements at five sites. There is apparantly significant difference in mono- and sesquiterpenes (t=11.621, p=0.00016).

381, p=0.001) and sesquiterpenes(F=6.713, p=0.0013) for all dates. These results suggested that essential oils on the leaf might be more volatile than the stem because leaves are expanded to easy to volatil-

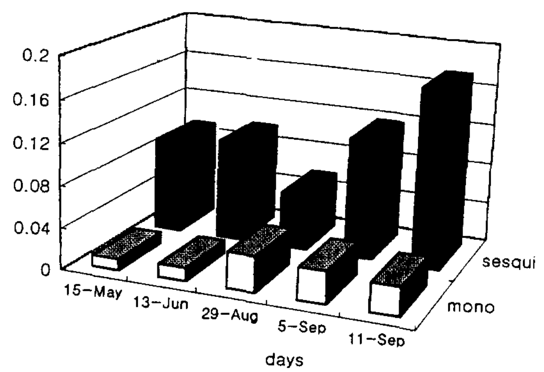

Fig. 4. The variation of the total concentrations between monoterpenes and sesquiterpenes in the stem of *C. boreale*. Means in each column come from five measurements at five sites. There is a significant difference in mono- and a significant difference in mono- and sesquiterpenes (t=4.19, p=0.0007).

ization. Most of sesquiterpenes are material for the biomedicine (Zygadlo *et al.* 1996), therefore *Chrysanthemum* species might be used traditional biomedicine by the presence of much amounts of sesquiterpenes in this plants.

A more detailed analysis of the complete leaf monoterpene and leaf sesquiterpene profile shows that the amounts of the individual constituents did not vary much among developmental stages (Table 1). I compared the developmental stages and the concentration of each terpenes by One-way ANOVA. These comparisons showed statistically significant differences ($p<0.05$) for two of the 13 leaf monoterpene compounds that comprise more than 5% of the total extract: (+)-Limonene (compound no. 7, R.T.=12.63) and unknown compound no. 13. An additional 13 monoterpenes were detected in average amounts of more than 5% for each dates. Artemisia princeps, the same family species with *C. boreale*, were detected 21 foliar monoterpenes, among of them α-pinene, β-pinene, β-myrcene, naphtalene and dl-limonene were present high concentrations (Kim 1996). The sesquiterpene fraction comprised large amounts of unidenti-

Table 1. The concentration (mg/g f.w.) of each leaf monoterpenes and sesquiterpenes with the time

Compound	R. T.	May 15	May 30	June 7	June 14	June 20	F-test	P value
Monoterpenes								
3 α-pinene	9.54	0.000385	0.002154	0.00188	0.003867	0.00337	0.301	0.874
4 (+)sabinene	10.54	0.001057	0.002707	0.003918	0.003029	0.001622	1.245	0.324
5 β-pinene	10.65	0.001087	0.001753	0.003485	0.002079	0.001634	0.687	0.6095
6 myrcene	12.40	0.000669	0.002277	0.001189	0.002306	0.003762	1.098	0.3845
7 (+)-limonene	12.63	0.000424	0.011969	0.015238	0.006989	0.003769	3.170*	0.036
8	12.84	0.000115	0.003932	0.006011	0.005027	0.003438	1.846	0.16
10	15.45	0.004763	0.018136	0.003362	0.006407	0.025452	2.171	0.109
11	16.09	0.000547	0.000302	0.002993	0.000688	0.000578	2.565	0.069
12	16.40	0.009583	0.000417	0.004576	0.000968	0.004583	1.472	0.248
13	17.28	0.001672	0.006598	0.000352	0.001869	0	3.189*	0.035
14	17.72	0.006271	0.00245	0.000808	0.015898	0.013073	1.286	0.309
15	20.25	0.005474	0	0	0.010107	0.001836	2.788	0.055
17	23.28	0.001717	0.004020	0.002031	0.002045	0.002417	2.215	0.104
Sesquiterpenes								
1 α-Humulene	24.54	0.019341	0.025904	0.024495	0.027836	0.028736	0.58	0.68
2	26.19	0.069764	0.115901	0.122848	0.120861	0.077892	2.398	0.0843
3	32.90	0.010469	0.006735	0.001734	0.005358	0.001482	1.236	0.327
5	34.12	0.01461	0.009735	0.00311	0.004884	0.01938	4.39	0.0104
6	34.60	0.001431	0.002509	0.001153	0.00061	0.00834	2.364	0.0877
7	35.04	0.001175	0.003009	0.003034	0.003229	0.007193	4.807*	0.007
9	35.71	0.001146	0.001938	0.004068	0.002666	0.012507	5.583*	0.0035

* means $p<0.05$
R. T. means Retention Time

fied compound no. 2 (R.T.=26.19) and α-Humulene (no. 1, R.T.=24.54). An additional 7 sesquiterpenes were detected in average amounts of more than 5% for each dates, there are two significant differences in seasonal variation of concentration in leaf sesquiterpenes : unknown compound no. 7 and compound no. 9 (Table 1).

The stem monoterpene fraction was dominated by α-pinene (no. 3, R.T. 9.54), β-pinene (no. 5, R.T. =10.65), no. 6 (R.T.=11.31), (+)-Limonene (no. 7, R.T.=12.63), myrcene and no. 14, and total 14 monoterpenes were detected in average amounts more than 5%. There are much significant differences in five monoterpenes concentration for developmental stages: compound no. 1, β-pinene, myrcene, compound no. 15 and no. 16 (Table 2). The sesquiterpene concentration of stem was dominated by α -Humulene (no. 1, R.T.=24.54) and unknown compound no. 2 (R.T=26.19), an additional 9 sesquiterpenes were detected in average amounts more than 5%. Similar to the results from the stem monoterpene, there are significant differences in the seven stem sesquiterpene for the developmental stages (Table 2). Goralka *et al.* (1996) reported that both of the monoterpenes total yield and composition varied with leaf maturity in *Umbellularia califonica*, however, only the composition of foliar monoterpene was varied, but the total concentration of foliar monoterpenes was not varied in this study.

The concentrational variation of each monoterpenes between leaf and stem was shown in Fig. 5. The monoterpenes in leaf and stem differ significant (t=3.

Table 2. The concentration (mg/g f.w.) of each stem monoterpenes and sesquiterpenes

Compound	R. T.	May 15	May 30	June 7	June 14	June 20	F-test	P value
Monoterpenes								
1	4.63	4.78×10^{-5}	0.001092	0.001526	0.000949	0.001602	8.282**	0.000414
2	4.96	0.000562	0.000653	0.000532	0.000615	0.001787	2.692	0.0607
3 α-pinene	9.54	0	0.006127	0.001439	0.00337	0.00322	1.223	0.3238
4 (+)sabinene	10.54	0	0	0	0.000473	0	1	0.4306
5 β-pinene	10.65	0	0.002977	0.003324	0.005117	0.004927	18.971***	1.39×10^{-6}
6 myrcene	12.40	2×10^{-5}	0.001053	0.001126	0.00152	0.001882	3.065*	0.0401
7 (+)-limonene	12.63	0	0.000711	0.003903	0.001315	0.000353	2.68	0.0613
9	14.30	0.000194	3.88×10^{-5}	0.010121	9.66×10^{-5}	0.000105	1.058	0.4027
10	15.45	5.6×10^{-5}	0.000352	0.000375	0.000144	0.000651	0.491	0.7423
12	16.40	0.001256	0	0.000677	0	0.000109	1.794	0.1696
13	17.28	0.001783	0	0	0	0	1	0.431
14	17.72	0.00047	0.000487	0.000516	0.0001812	0.001439	0.666	0.623
15	20.25	0.001021	0	0	0.00265	9.04×10^{-5}	4.03*	0.015
16	23.03	0.000923	0.000976	0.000978	0.001204	0.00119	3.094*	0.039
Sesquiterpene								
1 α-humulene	24.54	0.0245	0.01234	0.01424	0.02195	0.0230	3.321*	0.0306
2	26.19	0.0171	0.01137	0.00068	0.02215	0.01462	6.920*	0.0012
3	32.90	0.0018	0.00459	0.00016	0.00379	0.00295	1.497	0.241
4	33.25	0.00013	4.8×10^{-5}	0.00023	0	0.0075	11.553***	5×10^{-5}
5	34.12	0.01254	0.00818	0.00381	0.00704	0.02502	5.603*	0.0034
6	34.61	0.00204	0.00551	0	0.00165	0	3.103*	0.0386
7	35.04	0.00042	0.00559	0.00272	0.00488	0.00839	14.582***	9.95×10^{-5}
8	35.24	0.00132	0.00252	0.00121	0.00183	0.00302	2.079	0.122
9	35.72	0.00089	0.00286	0.00179	0.00505	0.00958	9.763**	0.00015

* means $p<0.05$

** means $p<0.001$

*** means $p<0.0001$

R.T. means retention times

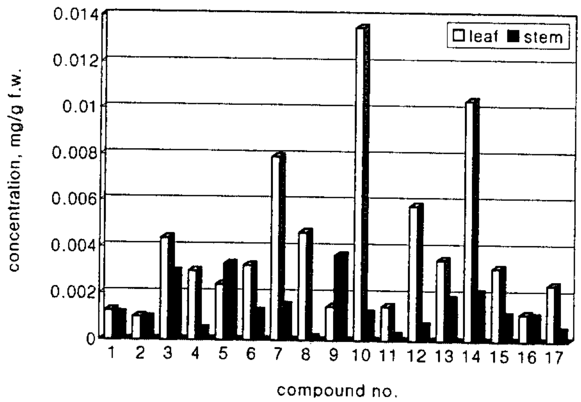

Fig. 5. The variation of mean concentration for each compounds of monoterpenes between leaf and stem of *C. boreale*. Each column represented that average obtained from standardized by five measurements belong to five sites, ie. 25 replications.

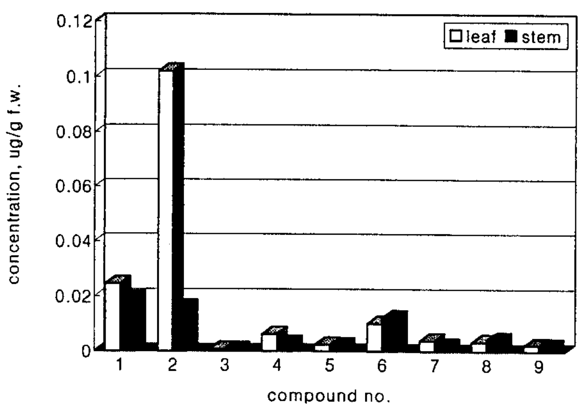

Fig. 6. The variation of mean concentraion for each compounds of sesquiterpenes between leaf and stem of *C. boreale*. Each data represented that average obtained from double standardized by five measurements at five sites, ie. 25 replications.

78, p=0.00072). However there is no significant difference in the yield of each sesquiterpenes between leaf and stem (t=1.38, p=0.183) (Fig. 6). This suggests that the concentrational variation of sesquiterpenes in both leaf and stem is less than monoterpenes, because the molecular weight of sesquiterpene is heavier than that of monoterpene, and therefore more difficult to move and volatilize. These results indicated that sesquiterpene concentrations were higher than monoterpenes of the leaf and stem in *C. boreale*, and thus sesquiterpenes are more important compound for the biomedicine in this plant. Many studies (Dubey and Kishore 1987, Croteau *et al.* 1981, Jimenez-Osornio *et al.* 1996, Zygadlo *et al.* 1996) suggested that several kinds of terpenes produced by many plants were shown to have important biological and ecological functions in nowadays. Although in this studies mentioned above, the main active compound was not identified, the study of medicinal and aromatic plants commonly used in traditional medicine, indicates that *C. boreale* is of fundamental importance for Korea. This chemical diversity is of particular interest in the selection of *C. boreale* for oil production.

적 요

산국 (*Chrysanthemum boreale*)의 잎과 줄기에 함유된 monoterpene과 sesquiterpene의 함량에 대한 초기 성장기 동안의 변이를 Gas Chromatography-Mass Spectrometry (GC-MS)를 이용하여 분석하였다. Sesquiterpene의 함량은 monoterpene함량보다 잎에서는 2.0~3.4배 가량 많았으며, 줄기에서는 1.6~8.3배 가량 높았다. 또한 잎에 있는 monoterpene과 sesquiterpene의 함량은 항상 줄기에 비해 높았으나, 잎의 성장에 따른 계절적 차이를 보이지는 않았으며, 줄기에 있는 monoterpene과 sesquiterpene의 함량은 계절적 차이를 나타내었다. Chromatogram상에서 보면, 약 17개의 monoterpene과 9개의 sesquiterpene이 인식되었으나 그 중 일부만이 동정되었고, 5% 이상의 존재를 나타내는 monoterpene은 잎과 줄기에서 각각 13, 14개이었고, 5% 이상의 존재를 갖는 sesquiterpene은 잎에서는 7개, 줄기에서는 9개이었다. 잎에서의 monoterpene 함량의 변이는 (+)-Limonene과 unknown compound no. 13 (R.T.=17.28)에서 뚜렷하였으며 sesquiterpene의 함량의 변이는 no. 7과 no. 9 두 개의 compound에서 나타났다. 줄기에서의 경우, monoterpene의 변이는 5가지의 monoterpene에서 나타났고 sesquiterpene의 변이는 7개의 compound에서 나타났으며 그 유의적 차가 매우 뚜렷하였다. 또한 sesquiterpene 가운데, 잎과 줄기에서 항상 α-Humulene과 unknown compound no. 2 (R.T.=26.19)가 많았다. 이상의 결과로부터, *Chrysanthemum*속에 속하는 식물들을 전통적인 민간의약으로 이용하는 하나의 이유는 그들이 함유하고 있는 sesquiterpene의 많은 양이기 때문

이란 것이 제시되었으며, 한국에 널리 퍼져 있는 산국에서의 terpene에 대한 화학적 함량의 변이가 이를 이용하는 분야에 기본적 자료를 제시할 수 있다고 판단된다. 비록 mono-와 sesquiterpene의 동정이 자세하게 이루어지지는 않았지만, 그들의 함량의 변이는 일차적으로 매우 유용하다고 판단된다.

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