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Extraction of essential oil components by cyclodextrins

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Liquid-liquid extraction using branched cyclodextrins (CDs) was studied for the purpose of modification of essential oil from trees. Compositions between original essential oil and the extractive using branchedCD were compared. The extractives by branched β-CD or branched γ-CD from hinoki leaf oil, sugi leaf oil or hinokiasunaro wood oil were higher of contents of β-eudesmol, cedrol or elemol, which has more miticidal activity, than original essential oils.

1. MATERIALS AND METHODS

1.1. Materials

6-O-α-maltosyl-α-CD (G_2-α-CD), 6-O-α-maltosyl-β-CD (G_2-β-CD), 6-O-α-maltosyl-γ-CD(G_2-γ-CD) and methyl-β-CD (Me-β-CD) were products of Ensuiko Sugar Refining Co.,Ltd. Hinoki leaf oil and sugi leaf oil were purchased from Amano Ringyo Co.,Ltd. and hinokiasunaro wood oil from Narita Ringyo-Doboku Co.,Ltd.

1.2. Extraction and analysis

To 5ml of 0.1M aqueous solution of a branched CD was added 1 g of an essential oil from tree and the mixture was stirred at 10,000 rpm for 10 min. Then the mixture was centrifuged at 3,000 rpm for 5 min to separate into 2 layers of aqueous branched CD solution layer and oil layer. One ml of the aqueous branched CD solution layer was shaken vigorously together with 40 ml of water, 20 ml of diethylether and 10 mg of n-tetradecane, an internal standard substance, to extract the oil components that transferred to the aqueous branched CD solution layer. The diethylether layer was analyzed by gas chromatograph (GC). GC was equipped with FID and was on 50m x 0.25 mm fused silica capillary coated column with TC-WAX. GC was programmed from 50℃ to 220℃ at 2℃/min. The pressure at inlet of carrier helium gas was set at 2 kgf/cm^2. The components of essential oils were identified by referring to the reports by B. Shieh et al.[1], by M. Yatagai et al.[2-3] and Okabe et al.[4] and to retention times of pure reagents of terpenes. Compositions of essential oils and the extractives by CDs were calculated from area ratio of the chromatograms.

1.3. Preparation of extractives by branched CDs

G_2-β-CD (290g) or Me-β-CD (260g) was dissolved in 2,000 ml of water, to which 120 g each of hinoki leaf oil, sugi leaf oil or hinokiasunaro wood oil were added. The mixture was stirred at 5,000 rpm at room temperature for 30 min.

After centrifuging at 3,000 rpm for 10 min, the mixture was filtered through a filter paper (Toyo No. 2) to recover the aqueous layer of CD solution. The aqueous CD solution layer was extracted 3 times with 500 ml each of diethylether. The diethylether layer was combined and its diethylether was allowed to evaporate under nitrogen gas stream. Twenty one grams of extracted oil fraction from hinoki leaf oil, 14 g from sugi leaf oil and 20 g from hinokiasunaro wood oil were obtained.

1.4. Estimation of miticidal activity

Original essential oils and the extractives by CDs were diluted with tetrahydrofuran to 5W/V% and 1W/V%, and 0.5 ml each of the diluted solutions was dropped uniformly over a filter paper (10 x 5 cm, Toyo No.5A). After drying in air for 1 hour the filter paper was folded in two and edge was fastened at 3 points with clips. Twenty mites (♀, adult) were confined between folded filter paper. The number of knocked-down mites was counted after 24 hours. To confirm death of mites, the knocked-down mites were transferred into a bag of intact filter paper and left at 25℃ and 75% of relative humidity for 24 hours. The number of mites that did not revive was made the number of killed mites. The rate of number of killed mites against total 20 mites used to the test was made the miticidal rate. Same test was repeated 3 times and the mean of results was taken as the final miticidal rate. As blank tests, similar procedures were conducted with untreated filter paper and with filter paper which was dropped 0.5 ml of tetrahydrofuran over and dried in air.

1.5. Evaluation of antimicrobial activity

Test microorganisms were Escherichia coli IFO 3301, Salmonella enteritidis IFO 3313, Staphylococcus aureus IFO 12732, Aspergillus niger IFO 4407 and Penicillium citrinum IFO 7784. Bacteria were cultured on Mueller Hinton Medium (Difco) and fungus on a potato-dextrose agar medium (Eiken Chemical).

To a plate medium was added a microbial suspension so as to contain 10^5 - 10^6 cells/ml, and over the surface was placed a paper disk, 8 mm in diameter, to which had been impregnated 50 μl of a successively double diluted solution with ethanol of an essential oil or extractive by CD. Bacteria were cultured at 35℃ for 24 hours and fungus at 25℃ for 7 days and the growth-inhibitory zone was examined. When a circular inhibitory zone with a diameter of longer than 10 mm was defined to be positive.

2. RESULTS AND DISCUSSION

2.1. Composition of extractives by CDs

The compositions between the extractive by CDs and the original essential oils were compared. Results of hinoki leaf oil, sugi leaf oil and hinokiasunaro wood oil are listed in Table 1, 2, 3, respectively.

Major components of hinoki leaf oil were sabinene, limonene, bornyl acetate, terpinyl acetate + α-terpineol and elemol. When G_2-α-CD was used an extractive whose

Table 1 Compositions of extractives by branched CDs from hinoki leaf oil.[b]

Compounds	Hinoki leaf oil	Extractive G_2-α-CD	G_2-β-CD	G_2-γ-CD
α-Pinene	2.3	7.3	0.1	0
Sabinene	14.3	12.3	1.7	0.3
Myrcene	5.5	1.7	0.1	0.2
Limonene	7.3	11.3	0.7	0.3
γ-Terpinene	3.9	0.3	0.1	0.2
Terpinolene	1.3	0	0.1	0.3
Bornyl acetate	6.8	12.6	7.6	8.9
4-Terpineol	3.4	17.2	13.8	17.1
Thujopsene	3.6	0	1.8	0.9
Terpinyl acetate + α-Terpineol[a]	17.6	16.2	12.9	9.3
Elemol	6.7	0.7	30.0	20.7
Total	72.7	79.6	68.9	58.2
Unknown	27.3	20.4	31.1	41.8

[a] *Terpinyl acetate and α-terpineol had the same retention time at this GC condition.*
[b] Percentages in the oils.

major components were sabinene, limonene, bornyl acetate, 4-terpineol, terpinyl acetate + α-terpineol were obtained. Major components of the extractive by G_2-β-CD were elemol, 4-terpineol, terpinyl acetae + α-terpineol. Use of G_2-γ-CD gave an extractive whose major components were elemol, 4-terpineol, terpinyl acetate + α-terpineol and bornyl acetate.

Major components of sugi leaf oil were kaurene, elemol, α-pinene and sabinene. When G_2-α-CD was used (+)-3-carene made up 37% and α-pinene 36% of total components of the extractive, while when G_2-β-CD was used elemol made up 36% and kaurene 31% of the extract components. Use of G_2-γ-CD gave an extractive containing 41% of elemol and 14% of β-eudesmol in the components.

Table 2 Compositions of extractives by branched CDs from sugi leaf oil.[a]

Compounds	Sugi leaf oil	Extractive G_2-α-CD	G_2-β-CD	G_2-γ-CD
α-Pinene	10.2	36.0	0.4	0.2
Sabinene	8.3	3.5	1.1	0.4
(+)-3-Carene	4.0	37.2	0.4	0.1
Myrcene	2.2	0.3	0	0.2
Limonene	5.3	4.4	0.4	0.5
γ-Terpinene	1.2	0	0	0.1
4-Terpineol	0.7	2.6	3.3	5.8
δ-Cadinene	2.7	0	0	0.4
Elemol	13.7	0	36.1	41.0
β-Eudesmol	6.4	0	7.4	14.4
Kaurene	17.5	1.2	31.2	0.5
Total	72.2	85.2	80.3	63.6
Unknown	27.8	14.8	19.7	36.4

[a] Percentages in the oils.

We could identify 8 components occupying 76% of hinokiasunaro wood oil. Thujopsene was contained as 62% of the total components.

Table 3 Compositions of extractives by branched CDs from hinokiasunaro wood oil.[a]

Compounds	Hinokiasunaro wood oil	Extractive G_2-α-CD	G_2-β-CD	G_2-γ-CD	Me-β-CD
Terpinolene	0.4	0	0	0	0.1
4-Terpineol	1.4	29.7	6.8	6.9	8.8
Thujopsene	61.5	0.8	21.4	10.3	3.6
α-Cuprenene	4.3	0	0.5	0.9	1.7
γ-Cuprenene	2.8	4.2	0.9	0.9	2.1
Cuparene	1.8	0	0.2	0.9	1.4
Cedrol	2.8	1.7	19.2	25.6	17.0
Widdrol	0.6	0	3.6	8.7	4.9
Total	75.6	36.4	52.6	54.2	39.6
Unknown	24.4	63.6	47.4	45.8	60.4

[a] Percentages in the oils.

Use of G_2-α-CD hardly gave an extractive from Hinokiasunaro wood oil. This was probably due to that G_2-α-CD did not form an inclusion complex with thujopsene. When G_2-β-CD, G_2-γ-CD or Me-β-CD was used, extractives whose cedrol and widdrol were concentrated were obtained.

Yatagai et al.[5] have reported that β-eudesmol and cedrol had strong miticidal activities (survival rates after 3 days at concentration of 8×10^{-3} mg/cm^2 were 15% and 37%, respectively) and elemol and α-terpineol had medium miticidal activities (survival rates after 3 days at concentration of 8×10^{-3} mg/cm^2 were 50-75%). In our present studies, elemol contents of the extractives by branched CDs were 3-4.3 times higher than that of hinoki leaf oil, at sugi leaf oil elemol and β-eudesmol contents of the extractives were 2.6-2.9 and 1.2-2.3 times, respectively, and at hinokiasunaro wood oil cedrol contents of the extractives

were 5.7-8.7 times.

2.2. Miticidal activity

The miticidal activities of the extractives by branched CDs and the original essential oils were measured. The results are listed in Table 4. The miticidal activity of the extractives by branched CDs was higher in parallel to the increased contents of elemol, β-eudesmol and cedrol than that of the original essential oils.

Table 4 Miticidal activity.

Extractives	Miticidal activity[a] 1 mg/cm²	0.2mg/cm²
Hinoki leaf oil	74.9%	0 %
Extractive by G_2-β-CD	91.7%	13.9%
Sugi leaf oil	91.4%	8.3%
Extractive by G_2-β-CD	98.3%	20.8%
Hinokiasunaro wood oil	62.2%	1.7%
Extractive by Me-β-CD	100.0%	10.0%

[a] The values of miticidal activity are shown at the miticidal rate.

2.3. Antimicrobial activity

The result is listed in Table 5. The oil fractions extracted by G_2-β-CD from hinoki and sugi leaf oils showed higher antibacterial activity against S. aureus in comparison with the original essential oils. The oil fraction extracted by Me-β-CD from hinokiasunaro wood oil showed quite a different antimicrobial spectrum from that of the original essential oil, with decreased activities against E. coli and S. enteritidis and increased activities against S. aureus and A. niger and particularly augmented activity against P. citrinum.

The above results show that liquid-liquid extraction using branched CDs can prepare oil fractions that contain some components in higher concentration than do the original essential oils from trees, and opened a possibility to obtain oil fractions afforded with qualities different from those of the original essential oils.

Table 5 Antimicrobial activity.

Microorganisms	Minimum inhibitory concentration[a] Oils[b] ①	②	③	④	⑤	⑥
E. coli	>100	>100	>100	>100	6.3	>100
S. enteritidis	>100	>100	>100	>100	12.5	>100
S. aureus	12.5	1.6	25.0	1.6	0.8	0.2
A. niger	>100	>100	>100	>100	50.0	12.5
P. citrinum	>100	>100	>100	>100	6.3	0.2

[a] The values of minimum inhibitory concentration are shown at percentage. >100 means no activity.
[b] Legend: ①:hinoki leaf oil, ②:extractive by G_2-β-CD from hinoki leaf oil, ③:sugi leaf oil, ④:extractive by G_2-β-CD from sugi leaf oil, ⑤:hinokiasunaro wood oil, ⑥:extractive by Me-β-CD from hinokiasunaro wood oil.

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