Volatile Components of the Kuromoji Essential Oil (Lindera umbellata Thunb.) and the Utilization for Touch Care Treatment

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Abstract: The volatile components of kuromoji oil (Lindera umbellata Thunb.) obtained in Shizuoka Pref. were analyzed by GC/MS. Linalool, α-pinene, limonene, camphene, cis- and trans-dihydrocarvone, 1,8-cineol, 4-terpinenol, α-terpinenol, piperitone, geranyl acetate, geraniol, and trans-nerolidol were identified as major components. Using enantio-MDGC-MS, the enantiomeric ratio ((R)-(+) vs (S)-(+)) of linalool in this oil was determined to be 67.8/32.2. Touch care treatment while sniffing this oil was done on cancer patients. We found that the relaxation effect persisted longer after the treatment compared to treatment without aroma.

Key words: kuromoji oil, Lindera umbellata Thunb., enantiomeric ratio, linalool, touch care treatment with aroma

1 Introduction

In the Futo district of Ito City, Shizuoka Prefecture, in 1940, nearly 1500 kg of the essential oil of kuromoji (Lindera umbellata Thunb.) were produced. This oil was used in exported perfumes and as an alternative to linalool as a solid soap fragrance¹⁻⁰. Recently, interest in kuromoji oil (KMO) and oobakuromoji oil (L. umbellata membranaeae), so called kuromoji oil, characteristic Japanese essential oils belonging to the Lauraceae family, has increased due to its psychological effects, which include stress reduction, stress regulation, and sedation¹⁻⁴. These effects are thought to be due to linalool, one of the major components of these oils.

It has been reported that the (R)-(-)-linalool enantiomer, but not the (S)-(+) enantiomer, is responsible for an analgesic effect of linalool¹⁷. In addition, only the (R)-enantiomer and racemates have sedative effects¹⁸. Other biological effects have been ascribed to the enantiomers¹⁰ and racemates of linalool, including anti-leishmanial activity in rac-linalool¹⁰, anesthetic activity in (S)-(+) linalool¹¹, and acaricidal activity in (R)-(−)-linalool¹². These enantiomers also have different fragrance properties¹³,¹⁴. Oobakuromoji oil and linalool also apparently induce apoptosis and differentiation in HL-60-cells¹⁵ and inhibit LPS-induced inflammation in vitro¹⁵.

Although the volatile constituents of KMO and oobakuromoji oil are similar, their odors differ. All of the kuromoji oils had relaxing and stress-reducing effects in healthy people who sniffed them for 5 min¹⁶. In addition, the anti-hypertensive, anti-inflammatory, and analgesic properties¹⁷ of Lindera plants apparently are due to linalool¹⁸. Few reports, however, have addressed the question of the chiralities of linalool in KMOs. For practical use of plant components and elucidation of plant physiology, determination of the ratios of enantiomers is important. For these reasons, we determined the enantiomeric ratio of linalool in KMO. We then studied the effects of sniffing this oil on touch care treatment (TCT) in cancer patients in our hospital and the effect was evaluated objectively using the heart rate variability (HRV)¹⁹⁻²³.

2 Materials and Methods

2.1 Materials

Kuromoji essential oil (KMO; L. umbellata Thunb.) manufactured in 2016 in Shizuoka Prefecture was purchased from Kaori no Haru Co. Ltd. and used for analysis of vola-
tiles and for touch care treatment (TCT). KMO was also used to determine the enantiomeric ratio of linalool. The two oobakuromoji oils (L. umbellata membranacea) were produced in Aomori Prefecture by Frangrance Journal Co. Ltd. and in Gifu Prefecture by Yuica Co. Ltd.

2.2 Methods

2.2.1 Instrumental analysis, GC/FID and GC/MS

Volatile components of KMO were identified by GC/FID and GC/MS, followed by comparison with the mass spectra and retention times of standards. Apparatus: Agilent HP6890 gas chromatograph. GC conditions: Column; 50 m × 0.25 mm × 0.15 μm BC-WAX (GL Sciences, Inc., Tokyo, Japan) and oven temperature increase from 70°C to 215°C at the rate of 4°C/min. The injector and ion source were set at 250°C and 200°C, respectively. The carrier gas was helium with a constant pressure set at 100 kPa.

2.2.2 Chiral GC analysis using enatio-MDGC-MS

The enantiomeric ratio of linalool in KMO was determined using MDGC-2010 (Shimadzu) coupled with a chiral column. An InertCap Pure-WAX column (50 m × 0.25 mm × 0.15 μm BC-WAX (GL Sciences)) was coupled to a β-DEX 325 chiral column (30 m × 0.25 mm × 0.15 μm; Supelco, Inc., Bellefonte PA, USA) as second column. For analysis of linalool, (R)-linalool and (S)-linalool standards were obtained from Aldrich Chemicals. The enantiomeric ratios were determined as described in Tomiyama et al.

2.2.3 Protocol of TCT and TCT with sniffing KMO

The protocol of TCT, which was the same as in previous reports, is shown in Fig. 1. The patient set up is illustrated in Fig. 2.

For TCT with aroma sniffing, KMO was first put into spherical silica gel beads to make an air care product (KMO 10 μg/Spherical silica gel beads 1.0 g) and placed into a sealed odor bag that then was attached to the patient’s chest before starting TCT. The subjects thus could smell the odor of KMO continually during TCT.

2.2.4 Ethics Review Board approval

This study was carried out under the approval of the Ethics Review Board of Shizuoka Cancer Hospital. The volunteers were lung cancer patients in our hospital who consented to participation in the study (N = 11, Female 1 and Male 10).

2.2.5 Evaluation of the effect of TCT with and without KMO using heart rate variability: Recording and analysis of autonomic parameters

Heart rate variability (HRV) and changes in autonomic nervous system (ANS) balance during TCT were measured using an HR sensor (myBeat®; Union Tool Co., Ltd.). To evaluate ANS activity, power spectral analysis on R-wave and R-wave intervals (RRI: temporal durations between beats) from the HR sensor was done. A small HR sensor (size: 4 cm², thickness 1 cm, weight 13 g) was attached to the subject’s chest and the HRV was recorded continually.

| Timetable | 5-10 min rest | 15-20 min before TCT | 5 min relaxation | TCT 10 min x 2 (20 min) | Rest after TCT 5 min | 15-30 min after TCT |
|-----------|---------------|----------------------|-----------------|------------------------|---------------------|-------------------|
| Measurement of ANS activity | | | | | | |
| Attachment (1) & Removal (2) of HR Sensor | (1) | | | (2) | | |
| Collection of saliva (3) | (3) | | | (3) | | |
| Questionnaire survey (4) | (4) | | | | | (4) |

Fig. 1 Protocol of TCT and TCT with aroma.

ANS: autonomic nervous system
TCT: touch care treatment; 20 min
TCT with aroma: TCT with aroma (5); ca. 22 min

Fig. 2 Illustration of TCT.
from the beginning to the end of the experiment. RRI and HR were calculated using an RRI Analyzer with analysis software from Union Tool Co., Ltd. To evaluate ANS balance, low frequency (LF; reflecting sympathetic and parasympathetic activities from 0.04 to 0.14 Hz) and high frequency power (HF; reflecting parasympathetic nervous system activities from 0.14 to 0.4 Hz) were used, and the LF/HF ratios were calculated.

2.3 Measurement of salivary stress markers

Two types of stress markers in saliva, \( \alpha \)-amylase (\( \alpha \)-Amy) and chromogranin A (CgA) were measured in patient saliva collected before and after TCT.

2.3.1 Measurement of \( \alpha \)-amylase activity

Patient saliva was collected before and after the operation and used as analytical samples. \( \alpha \)-Amy in saliva before and after treatment was calculated using a Salivary \( \alpha \)-Amylase Assay Kit in accordance with the manufacturer’s instructions (Salimetrics Ltd.).

2.3.2 Measurement of CgA

Saliva obtained in the same manner as described above was stored at \(-30^\circ C\) and thawed to room temperature \((25^\circ C)\) in a water bath before use. CgA was determined using the YK070 Human Chromogranin A EIA, employing methods developed in the laboratory of Yanaihara.

3 Result and Discussion

3.1 Volatile components of KMO (\( Lindera umbellata \) Thunb.)

As shown in Table 1, linalool, \( \alpha \)-pinene, camphene, limonene, \( cis \)- and \( trans \)-dihydrocarvone, 1,8-cineol, 4-terpinenol, \( \alpha \)-terpineol, piperitone, geranyl acetate, geraniol, and other compounds were identified in KMO. The table below shows the details of the volatile components:

| Compound | RT (min) | RI (BC-WAX) | Futo | Izu Nagoya | Osaka | Aomori |
|----------|----------|-------------|------|------------|-------|--------|
| \( \alpha \)-pinene | 2.64 | 1026 | 3.87 | 1.43 | 7.11 | 0.22 |
| Fenchene | 2.83 | 1056 | 0.04 | | | |
| Camphene | 2.85 | 1095 | 2.53 | 4.41 | 0.16 | |
| \( \beta \)-pinene | 2.86 | 1119 | 0.91 | 0.12 | 1.31 | |
| 3-carene | 3.14 | 1130 | 1.08 | 1.78 | | |
| Myrcene | 3.28 | 1148 | 0.65 | 0.13 | 7.68 | |
| Phellandrene | 3.62 | 1192 | 0.93 | | 0.16 | |
| Limonene | 3.86 | 1207 | 4.81 | 6.71 | 1.09 | 7.42 |
| 1,8-cineol | 3.97 | 1220 | 16.75 | 18.21 | 30.61 | 24.51 |
| Ocimene | 4.13 | 1221 | 0.12 | | 5.01 | |
| \( \alpha \)-terpinolene | 4.18 | 1228 | 1.05 | | | |
| Terpinene | 4.21 | 1232 | 2.03 | | | |
| 4-cymene | 4.41 | 1258 | 1.37 | | 1.26 | |
| \( iso \)-terpinolene | 4.45 | 1260 | 0.11 | | | |
| Terpinolene | 4.51 | 1270 | 0.53 | | | |
| \( iso \)-amyl iso-valerate | 4.62 | 1287 | 0.11 | 0.61 | | |
| \( iso \)-prenyl iso-varlate | 5.13 | 1357 | 0.06 | | | |
| \( Z \)-3-hexenol | 5.19 | 1366 | 0.08 | 0.23 | | |
| \( trans \)-linalool-3,6-oxide | 5.63 | 1428 | 0.68 | 0.14 | | |
| Sabinene hydrate | 5.75 | 1447 | 0.06 | | | |
| \( cis \)-linalool-3,6-oxide | 5.81 | 1457 | 0.54 | | | |
| Camphor | 6.13 | 1506 | 0.67 | | | |
| Linalool | 6.27 | 1529 | 26.83 | 31.91 | 28.61 | 28.84 |
| \( R \)-linalool* | 6.27 | 1529 | 18.21 | | 42.8 | |
| \( S \)-linalool* | 6.27 | 1529 | 8.62 | | | |
| \( iso \)-bornyl acetate | 6.52 | 1570 | 1.49 | | 1.46 | |

J. Oleo Sci. 70, (11) 1661-1668 (2021)
and \textit{trans}-nerolidol were identified as the major components of KMO. These were similar to those reported in oils obtained in the Izukogen and Izu Nagaoka areas,\textsuperscript{29,30} Shizuoka Pref., and in Osaka Pref.\textsuperscript{31} The contents of linalool and 1,8-cineol were different than those found in oobakuromoji oil from Aomori Pref.\textsuperscript{5,32}.

### 3.2 The enantiomeric ratio of linalool in KMO

The content of $(R)$- and $(S)$-enantiomers of linalool in KMO described in Table 1 were calculated from peak areas in GC and GC-MS analyses. The enantiomeric ratio of linalool in KMO used for TCT was 67.8/32.2, and then $(R)$-$(S)$-enantiomer was included a little more shown in Fig. 3.

These results were very interesting compared with the data of oobakuromoji oils (\textit{L. umbellata} membranacea) produced in Aomori\textsuperscript{5} and Gifu Pref.\textsuperscript{6} (Table 2).

### 3.3 Evaluation of the effect of TCT with KMO aroma

#### 3.3.1 Changes of HR values

During TCT with and without aroma, HR values decreased from initial levels before treatment by 2-3 beats/min (Fig. 4). HR decreased from 75.3 (initial) to 73.7 (during TCT), and then increased after TCT to a level almost identical to the initial value of 75.2 (beats/min) (Fig. 4, dotted line). In contrast, while sniffing KMO, HR decreased from 75.0 (initial) to 72.0 during TCT and remained at 72.0 after treatment had ended (Fig. 4, solid line). All patients displayed these trends.

In a previous study on aromatherapy\textsuperscript{17}, when healthy persons smelled KMO for 5 min, HR values decreased by

\begin{table}[h]
\centering
\caption{Continued.}
\begin{tabular}{lcccccc}
\hline
\textbf{Compound} & \textbf{RT (min)} & \textbf{RI (BC-WAX)} & \textbf{Futo} & \textbf{Izukogen}\textsuperscript{1)} & \textbf{Izu Nagaoka}\textsuperscript{29,30)} & \textbf{Osaka}\textsuperscript{31)} & \textbf{Aomori}\textsuperscript{32)} \\
\hline
\beta\text{-}elemene & 6.59 & 1581 & 0.35 & & & & 1.74 \\
4-terpinenol & 6.64 & 1589 & 3.71 & & & & \\
linalool-3,7-oxide acetate & 6.77 & 1611 & 0.08 & & & & \\
\textit{trans}-dihydrocarvone & 6.79 & 1608 & 1.91 & & & & 0.26 \\
aromadendrene & 6.71 & 1602 & 0.13 & & & & \\
cis-dihydrocarvone & 6.83 & 1620 & 1.81 & & & & \\
citronellyl acetate & 6.99 & 1647 & 0.04 & & & & \\
humulene & 7.11 & 1669 & 0.14 & & & & \\
selinane & 7.18 & 1675 & 0.27 & & & & \\
\alpha\text{-}terpineol & 7.23 & 1685 & 3.35 & & & & 4.3 1.46 \\
borneol & 6.91 & 1686 & 0.66 & & & & 0.5 \\
\alpha\text{-}selinene & 7.35 & 1711 & 0.72 & & & & \\
germacrene D & 7.36 & 1712 & 0.13 & & & & \\
piperitone & 7.37 & 1717 & 1.69 & & & & 0.39 \\
\beta\text{-}bisabororene & 7.45 & 1727 & 0.11 & & & & \\
\alpha\text{-}farnesene & 7.47 & 1737 & 0.18 & & & & \\
geranyl acetate & 7.51 & 1741 & 10.45 11.41 1.31 1.02 & & & & \\
norol & 7.72 & 1779 & 0.13 & & & & 0.62 \\
dihydrocarveol & 7.78 & 1888 & 0.08 & 3.88 & & & \\
geraniol & 7.97 & 1822 & 4.51 7.31 1.31 0.97 & & & & \\
\textit{trans}-nerolidol & 8.98 & 2024 & 1.36 3.31 3.42 & & & & \\
thymol & 9.69 & 2170 & 0.13 & & & & \\
\beta\text{-}eudesmol & 9.87 & 2208 & 0.15 & & & & \\
\hline
\end{tabular}
\end{table}

\[\text{Izukogen, Izu Nagaoka, Futo in Shizuoka Pref.}
\]
\[\text{Futo: used in this study}
\]
\[\text{Osaka: data was cited from leaves of kuromoji}
\]
\[\text{Aomori: oobakuromoji (ref. 32)}
\]
\[\text{ref. 1), ref. 29-32); see References}
\]
\[\text{* calculated from GC area (%) and MDGC-MS area (%)}
\]
only 0.5-1.0 (from 69 to 68.5). This decrease was ascribed to aromatherapy. However, long-term TCT experiments were not conducted, so the lasting effects of treatment remain unknown. In another study, TCT was performed with healthy people and cancer patients to reduce stress and decrease pain\(^3\), but changes in HR were not reported.

#### 3.3.2 Effects of aromatherapy on HF and LF/HF ratio

To determine the patients’ ANS status, we focused on low (LF) and high (HF) frequency power. In patients treated with KMO during TCT, HF values increased from 89.0 (initial) to 177 (during treatment), reaching 230 ms\(^2\) after treatment (Fig. 5). In contrast, patients who did not receive aromatherapy showed initial HF values of 65.1 that increased during treatment to 73.9, and then decreased to a final value of 62.5. LF/HF ratio in patients undergoing TCT with KMO remained relatively constant at a level of 2.0 (Fig. 6, solid line), but increased from 2.3 to 3.0 (Fig. 6, dotted line) in untreated patients.

These data show that parasympathetic activity of lung cancer patients was enhanced during TCT while sniffing the aroma of KMO. This treatment caused a reduction in HR relative to HR in untreated patients and this reduction remained after treatment. TCT with KMO thus has a relaxing effect in cancer patients. This effect could be further evaluated using HRV.

#### 3.4 Stress markers in saliva (\(\alpha\)-amy and CgA)

\(\alpha\)-Amy activity in saliva decreased from 369 to 300 KU/L during TCT. Addition of aroma did not change these results significantly (Fig. 7).

Some differences were observed with CgA. During TCT with no aroma, values changed from 4.7 (before treatment) to 4.8 (after treatment). However, in the KMO treatment group, CgA decreased from 6.0 to 5.0 (pmol/mg protein) (Fig. 8). Whether KMO treatment had significant effects thus remains unclear.

#### 4 Conclusion

The major components of KMO were found to be linalool, \(\alpha\)-pinene, limonene, camphene, cis- and trans-dihydrocar-

| Essential Oil                  | Production Area | (R)-(−) | (S)+(+) |
|-------------------------------|-----------------|---------|---------|
| KMO L. umbellata Thunb.       | Shizuoka        | 67.8    | 32.2    |
| oobakuromoji L. umbellata membranacea | Gifu          | 14.6    | 85.4    |
| oobakuromoji L. umbellata membranacea | Aomori      | 39.9    | 60.1    |
vone, 1,8-cineol, 4-terpinenol, α-terpineol, piperitone, geranyl acetate, geraniol, and trans-nerolidol. The enantio-
meric ratio of (R)-(-)- to (S)-(+)-linalool in KMO was 67.8/ 32.2. By studying TCTs with and without KMO in cancer 
patients, we conclude that parasympathetic nervous 
system (PNS) activity was enhanced by the KMO aroma. TCT with KMO had a relaxing effect that persisted longer 
than TCT without aroma. The number of patients was low, 
at 11, of whom 10 were male. Therefore, to better validate 
our results, additional patients should be tested.

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Conflict of Interests
There are no conflict of interests in this study.

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