TRADITIONAL & KAMPO MEDICINE

ORIGINAL ARTICLE

Antidepressive-like effect of volatile components of kososan in a mouse model of stress-induced depression

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ABSTRACT

Aim: Kososan (Xiang-Su-San in Chinese), a kampo (traditional Japanese herbal) medicine, contains large amounts of unique volatile components (termed KSv). This study evaluated the antidepressive-like effects of KSv alone or in combination with the water-soluble extracts of kososan (termed KSw), neither of which is fully known, in a mouse model of stress-induced depression.

Methods: KSv, KSw + KSv (in toto or with a specific component herb removed), or KSw + perillaldehyde (a major volatile component of perillae herba) was given to stress-induced depression-like model mice for 9 days, followed by measurement of immobility time in forced swimming test (FST). KSv was also analyzed on gas chromatography–mass spectroscopy.

Results: KSv (100%, inhaled) significantly reduced FST immobility time. KSw (0.5 g/kg, oral) + KSv (50%, inhaled) reduced immobility time to a significantly greater extent than did either alone, whereas KSw + KSv with perillae herba removed (50%, inhaled) did not. Likewise, KSw + 0.5% (v/v) perillaldehyde significantly had less immobility time than did either alone.

Conclusion: Inhaled KSv has antidepressive-like activity in a mouse model of stress-induced depression and enhances the antidepressive-like activity of KSw. Perillaldehyde in perillae herba may account in part for the enhancing effects of KSv.

KEY WORDS: antidepressive-like activity, depression, kampo medicine, kososan, perillaldehyde, volatile component

INTRODUCTION

Kososan (Xiang-Su-San in Chinese) is a kampo (traditional Japanese herbal) medicine composed of five herbs: cyperi rhizoma, perillae herba, aurantii nobilis pericarpium, glycyrrhizae radix, and zingiberis rhizoma. It has been used clinically to treat depression along with the initial stage of the common cold, allergic urticaria due to food ingestion, irritable bowel syndrome, chronic fatigue syndrome, insomnia, and autonomic imbalance. There is evidence showing that the water-soluble extract of kososan (KSw) alleviates depressive mood caused by interferon-α (IFN-α) treatment for hepatitis C [1]. Moreover, our previous studies demonstrated that oral KSw counteracted the depression-like behavior of stressed or IFN-α-treated mice by restoring hypothalamic-pituitary-adrenal (HPA) axis function, which is strongly associated with the pathogenesis of depression [2,3]. As suggested by our subsequent study, regulation of the orexinergic system by long-term KSw plays an important role in its antidepressive-like effect in stress-induced model mice [4,5]. These findings indicate that KSw is beneficial for treating depression.

Unlike Western drugs, kampo medicines have unique odors derived from their component herbs. When patients decoct kampo medicines themselves, they inhale the odor from the decoction, which may have pharmacological effects; therefore, kampo medicines may act via the olfactory nervous system as well as orally. Lemon oil vapor has antidepressive properties in rats [6] and exerts anxiolytic and antidepressive-like effects in mice by modulating the

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dopaminergic and serotonergic activities [7]. The odor emanating from green leaves, which is due to 6-carbon aliphatic alcohols and aldehydes, also reduces depressive-like behaviors by altering the serotonergic system [8]. In addition, our previous study showed that inhaling perillaldehyde, a major component of the essential oil in perillae herba, attenuates the depression-like symptoms of stressed mice [9]. Collectively, these findings suggest that olfactory stimulation by specific odors is a promising means of treating depression.

Among the herbs comprising kososan, cyperi rhizoma, perillae herba, and aurantii nobilis pericarpium contain large amounts of volatile components such as essential oils. Whether inhalation of the volatile components of kososan (KSv) reduces depression, however, is unclear. It is possible that the combined effect of volatile components and water extracts of kampo medicines is a characteristic of the pharmacological activity of kampo medicines.

In the present study, to address this issue, we examined the effects of KSv on the depression-like behavior of stressed mice using the forced swimming test (FST). We also assessed the combined effects of oral KSw and inhaled KSv in this mouse model.

METHODS

Animals

Adult (7-week-old) male ddY mice (Japan SLC, Hamamatsu, Japan) weighing 35–40 g were used in the experiments. The mice were housed individually at a constant temperature (23 ± 2°C), humidity (55 ± 10%), and light–dark cycle (12/12 h; 8:00–20:00 hours). Food and water were available ad libitum unless otherwise specified. The present study was approved by the Institutional Animal Care and Use Committee of Kitasato University, and all animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals of Kitasato University. Every effort was made to minimize the number of animals used and their suffering.

Drugs

The herbs comprising kososan were as follows: cyperi rhizoma (the rhizome of *Cyperus rotundus* L.), 4.0 g (lot no. AE7951, Tsumura, Tokyo, Japan); perillae herba (leaf of *Perilla frutescens* Britton var. acuta Kudo), 2.0 g (lot no. B04401, Tsumura); aurantii nobilis pericarpium (pericarp of *Citrus unshiu* Markovich), 3.0 g (lot no. AD7971, Tsumura); glycyrrhizae radix (root of *Glycyrrhiza uralensis* Fisher), 2.0 g (lot no. 8661621, Uchida Wakan-yaku, Tokyo, Japan) and zingiberis rhizoma (rhizome of *Zingiber officinale* Roscoe), 0.5 g (lot no. AK8761, Tsumura). The kampo formula was decocted with 600 mL distilled water until the volume was reduced to half. The extract was immediately filtrated under vacuum and the filtrate was lyophilized. The yield of KSw was approximately 28% of the herbal mixture, based on dry weight [2–5,10,11].

KSv and the volatile components of kososan with cyperi rhizoma removed (KSv–CR), kososan with perillae herba removed (KSv–PH), and kososan with aurantii nobilis pericarpium removed (KSv–ANP) were prepared using an essential oil quantification apparatus and a reflux condenser. Briefly, each formulation was decocted in 600 mL distilled water for 6 h, and each volatile component captured in the apparatus was collected (approximately 0.3 mL each). The components of KSv were identified on gas chromatography–mass spectroscopy (GC/MS). Mass spectra were recorded on an HP5970 (Hewlett-Packard, Palo Alto, CA, USA) coupled to a HP5980B (Hewlett-Packard) with an SP-2380 column (0.25 mm i.d. × 30 m, 0.25 μm film thickness; Aldrich-Supelco, St Louis, MO, USA). GC injector temperature was 250°C. The oven temperature was programmed to 60°C and held for 1 min, and then progressively increased to 100°C by 30°C/min, then to 160°C by 1°C/min, and then to 200°C by 30°C/min, and held at 200°C for 5 min. MS was carried out under the following conditions: electron impact source of 70 eV, 250°C. Identification of peaks was made according to retention time and the MS spectrum using a standard compound. Perillaldehyde as a standard compound was purchased from Aldrich Chemical (St Louis, MO, USA). Additionally, KSv and KSw were analyzed on high-performance liquid chromatography (HPLC) to assess differences in their constitutional patterns according to our previous methods [2,3].

Drug treatment

Freeze-dried KSw was dissolved in distilled water and administered orally to mice (0.5/kg, 0.5 mL/mouse, once daily for 9 days; Fig. 1). Although our previous study showed that KSw (1.0 g/kg) reduced depression in mice with stress-induced depression-like behavior [2], we used a lower, ineffective dose (0.5 g/kg) when determining its effects in combination with other drugs. KSv was diluted in 10%, 50%, or 100% (v/v) with ethanol; KSv–CR, KSv–PH, and KSv–ANP were diluted in 50% (v/v) with ethanol; and perillaldehyde was diluted in 0.2%, 0.5%, or 1% (v/v) with ethanol. To allow mice to smell the volatile components and

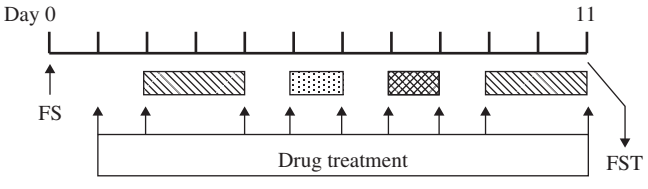

Figure 1 | Experimental design for inducing chronic mild stress (CMS); and drug treatment. (▨) Cage tilting (CMS 1); (▩) dirty bedding (CMS 2); (▩), cage shaking (CMS 3). FS, forced swimming; FST, forced swimming test.

perillaldehyde, the solutions were dropped between their nose and eyes (5 and 10 μL/mouse, respectively) once daily for 9 days (Fig. 1) [9].

Stress-induced depression-like model mice

Mice with stress-induced depression-like behavior were prepared by a combination of modified forced swimming [12,13] and application of chronic mild stress (CMS) [14,15] as previously described [2]. Briefly, the mice were individually placed in separate 5 L glass beakers (height, 27 cm; diameter, 18 cm) filled with 4 L of water (23 ± 1°C) for 15 min on day 0. The mice were then removed and dried with a hot-air dryer before being returned to their home cages. After 2 days, the mice were exposed to three different stress situations: tilting of the cage 30° from horizontal on days 2 and 9 (CMS 1); pouring of 200 mL of water into the sawdust bedding of the cage on day 5 (CMS 2); and shaking of the cages at 200 r.p.m. using a Green S. Seriker II apparatus (Vision Scientific, Kyunggi, Korea) on day 7 (CMS 3). These stresses were applied for 48, 24, and 24 h, respectively, with 24 h intervals. On day 11, the mice were then placed in 5 L beakers filled with 4 L of water 1 h after the final cage tilting, and FST was performed for 5 min (Fig. 1). The total duration of immobility during 5 min FST was measured 60 min after the final KSw or KSv treatment. FST was conducted between 13:00 and 16:00 hours.

Statistical analysis

Results are presented as mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by Tukey's test with StatView 5.0 (SAS Institute, Cary, NC, USA). Differences were considered significant at $P < 0.05$.

RESULTS

Effect of KSw, KSv on duration of FST immobility

Stress significantly increased the duration of immobility compared with control (no stress) conditions, and inhalation of 100% KSv significantly inhibited the stress-induced increase (Fig. 2a). The combination of oral KSw (0.5 g/kg) and 50% KSv also significantly inhibited the stress-induced increase in immobility time, whereas either alone did not (Fig. 2b).

Unlike the combination of KSw (0.5 g/kg, oral) and KSv (50%, inhaled), the combination of KSw and KSv–PH did not significantly inhibit the stress-induced increase in immobility time (Fig. 2c). Immobility time was slightly but not significantly reduced by KSw + KSv–ANP or KSv–CR.

KSv GC/MS profile

The major component of KSv was identified on GC/MS. The GC retention time (Fig. 3b) and mass spectrum position (Fig. 3d) of the largest peak in the KSv were the same as those of the perillaldehyde standard (Fig. 3a,c). Therefore, the major component of KSv is perillaldehyde (Fig. 3b).

Effect of perillaldehyde on duration of FST immobility

Inhalation of 1% perillaldehyde significantly inhibited the stress-induced increase in immobility time (Fig. 4a), in agreement with the results of our previous study [9]. The combination of oral KSw (0.5 g/kg) and inhaled perillaldehyde (0.5%) also inhibited this increase compared with KSw or perillaldehyde alone, neither of which had an effect (Fig. 4b).

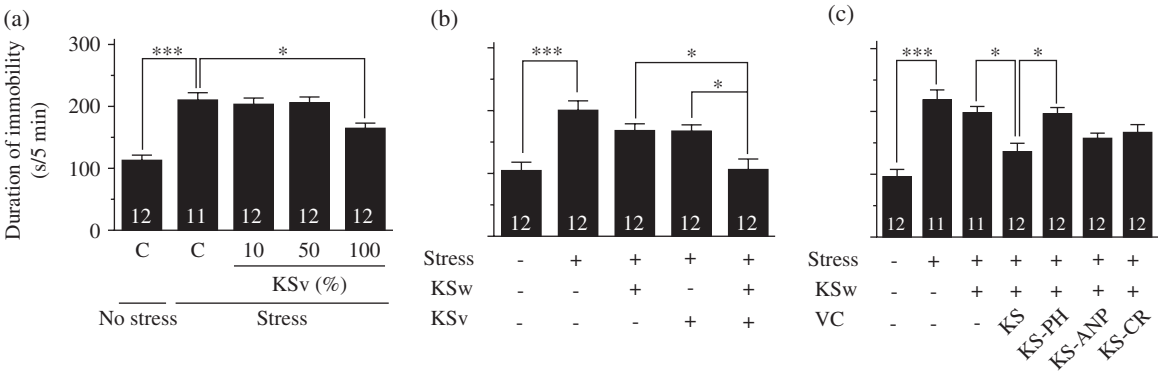

Figure 2 | Effect of (a) volatile components of kososan (KSv); (b) combination of the water-soluble (KSw) and KSv components of kososan; and (c) KSw and KSv either in toto or with perillae herba, aurantii nobilis pericarpium, or cyperi rhizoma removed (KSv–PH, KSv–ANP, and KSv–CR, respectively) on the duration of immobility in the forced swimming test in the stress-induced depression-like model mice. Mice received (a) KSv (10%, 50%, or 100%) via inhalation; or (b) KSw (0.5 g/kg, oral) and/or KSv (50%, inhaled); or (c) oral KSw (0.5 g/kg, oral) and/or KSv, KS-PH, KSv–ANP, or KSv–CR (each 50%, inhaled) for 9 days, and immobility time was measured 60 min after the final treatment. Each column represents the mean ± SEM. n, no. mice per treatment. *$P < 0.05$, ***$P < 0.001$ (Tukey test). C, vehicle-treated control; VC, volatile component.

DISCUSSION

Inhalation of KSv reduces depression in a mouse model of stress-induced depression (Fig. 2a) and enhances the antidepressive-like activity of oral KSw (Fig. 2b). We also found that these effects are mediated by perillaldehyde, which is derived from perillae herba, a major constituent of KSv (Fig. 3). Therefore, the present study provides an important insight into the pharmacological effects of a mixture of the volatile and water-soluble components of kososan.

To assess the combined effect of KSw and KSv, mice were treated with KSw (0.5 g/kg, oral), KSv (50%, inhaled), or both. Neither alone had antidepressive-like activity, whereas both together did (Fig. 2b) [2]. This suggests that KSw and

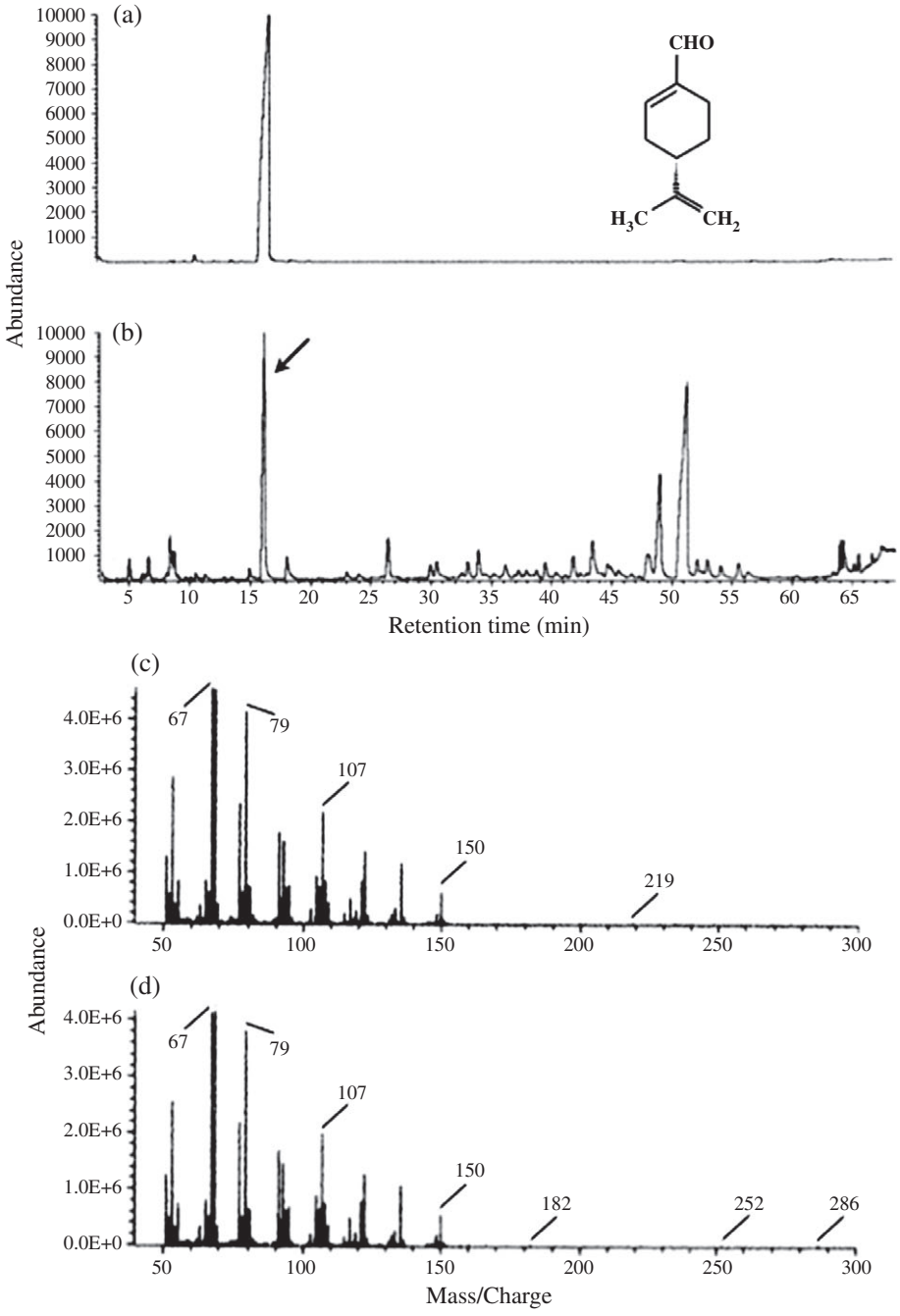

Figure 3 | Gas chromatography–mass spectroscopy profiles of the volatile components of kososan (KSv). (a,b) Gas chromatography profiles of (a) perillaldehyde and (b) KSv. (a) Chemical structure of perillaldehyde. (c,d) Mass spectroscopy profiles of (c) perillaldehyde and (d) the peak (arrow in b) of the KSv.

KSv counteract depression synergistically. The aroma of lemons has been reported to reduce depression and to potentiate the effects of the tricyclic antidepressant imipramine in rats [6]. Moreover, a clinical study demonstrated that exposure of humans to a citrus fragrance markedly decreased the doses of antidepressants required for treating depression [16]. These findings, as well as the present ones, provide strong evidence of the collaborative antidepressive-like actions of orally administered drugs and olfactory stimulation.

To identify the key herb(s) involved in the antidepressive-like effect of KSv, we removed specific herbs from KSv. KSv–PH did not enhance the antidepressive-like activity of KSw, whereas KSv–ANP and KSv–CR had a slight but insignificant effect (Fig. 2c). This indicates that enhancement requires the volatile components of perillae herba and perhaps those of aurantii nobilis pericarpium and cyperi rhizoma as well. This may be supported by the finding that the lemon odor, which contains limonene (also in aurantii nobilis pericarpium) as a major ingredient, has an antidepressive-like property [6], whereas the relevant odor in cyperi rhizoma is unknown. The present results also imply that a cocktail effect of the volatile components derived from the component herbs may play a synergistic role in the enhancement of the antidepressive-like effect. Further studies are needed to assess the relationship between the volatile components of aurantii nobilis pericarpium and cyperi rhizoma and the enhancement of antidepressive-like effects.

Component analysis of KSv on GC/MS identified perillaldehyde, a major component of an essential oil in perillae herba, as the major source of the antidepressive-like activity of KSv (Fig. 3). In addition, it was confirmed on HPLC that perillaldehyde is present in KSv, but not in KSw (Figure S1). Our previous study showed that inhalation of 1% perillaldehyde had an antidepressive-like activity in the mouse model of stress-induced depression [9]. Therefore, it seems likely that perillaldehyde is the perillae herba ingredient that primarily enhances the antidepressive-like effect of KSw. In support of this premise, we found that KSw (0.5 g/kg, oral) + perillaldehyde (0.5%, inhaled) alleviated the depressive-like behavior of stressed mice, whereas either alone did not (Fig. 4). Consistent with our previous study [9], 1% perillaldehyde alone had an antidepressive-like activity in this mouse model (Fig. 4a). Although the present results identify perillaldehyde as the enhancing component of KSv, we note that additional volatile components may also have enhancing effects, as suggested by the GC profiles in Figure 3(b). Identification of these other components will further our understanding of how KSv augments the antidepressive-like activity of KSw.

Our previous study showed that the antidepressive-like effect of KSw (1.0 g/kg, p.o.) was mediated by suppressing the stress-induced hyperactivity of the HPA axis in mice [2]. It has also been implied that perillaldehyde (dose of 10%, inhalation) has an inhibitory effect on hyperactivity of the HPA axis (Ito N, 2008, unpublished data). Although the precise mechanisms underlying the antidepressive-like effect of KSv are still unclear, we speculate that the antidepressive-like activity of KSv and a combination of KSv and KSw might be mediated partly through improvement of dysfunction of the HPA axis. Further studies are required to clarify the underlying mechanisms.

The present study had two limitations. First, the volume of the volatile components collected by decocting kososan for 6 h was very small (approx. 0.3 mL) because not all volatile components were collected via this method. To precisely quantify the total amount of the volatile components, a more efficient extraction procedure is needed. Second, it is unclear whether KSw and KSv act in combination in mice with olfactory dysfunction (i.e. anosmia). Clinical studies suggest that olfactory impairment is a potential problem in patients with major depression [17]. Therefore, the combined effect

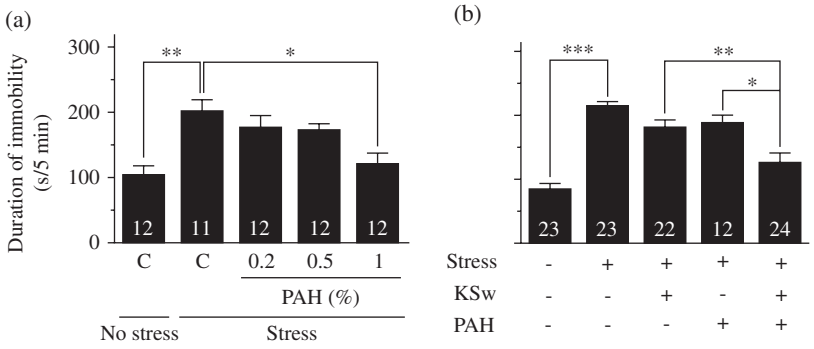

Figure 4 | Effect of (a) perillaldehyde and (b) combination of the water-soluble component of kososan (KSw) and perillaldehyde on the duration of immobility in the forced swimming test in the stress-induced depression-like model mice. Mice received (a) perillaldehyde (0.2%, 0.5%, or 1%) via inhalation or (b) KSw (0.5 g/kg, oral) and/or perillaldehyde (0.5%, inhaled) for 9 days, and immobility time was measured 60 min after the final treatment. Each column represents the mean ± SEM. n, no. mice per treatment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Tukey test). C, vehicle-treated control; PAH, perillaldehyde.

of KSw and KSv may not occur in depressed patients with impaired olfactory function. But, although there is no evidence that the mice in the present model are prone to anosmia, the issue may be solved by examining the combined effect of KSw and KSv using olfactory bulbectomized animals, a well established animal model of depression [18]. Thus, the present results highlight the potential benefits of the combination of KSw and KSv for treating depressive-like behaviors in mice with intact olfaction.

In conclusion, this study provides the first evidence that inhaled KSv has an antidepressive-like effect per se and synergistically enhances the antidepressive-like activity of KSw in a mouse model of stress-induced depression. Perillaldehyde, a component of perillae herba, may be the major volatile component responsible for synergy. Because numerous kampo medicines possess unique volatile components, unlike existing antidepressants, the present findings suggest that the odors of kampo medicines might potentiate their oral effects. Perillaldehyde may be useful for reducing the dosage of antidepressants without compromising their effectiveness. This might reduce the adverse effects that occur during long-term treatment of depression, and warrants further examination.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 | High-performance liquid chromatography profiles of (A) KSv and (B) KSw. Peaks of perillaldehyde and *m*-xylene, in which KSv was dissolved, are indicated by an arrowhead and arrows in A, respectively. Note that KSw had very little volatile component.