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Extraction of Essential Oils of Lemon Grass Using Ionic Liquid

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Geranial [(*E*)-3,7-dimethyl-2,6-octadienal], neral [(*Z*)-3,7-dimethyl-2,6-octadienal] and geraniol [(*E*)-3,7-dimethyl-2,6-octadien-1-ol] are found in lemon grass and are attractive essential oils with potential medicinal applications. In this study, a new method for extracting these natural flavour substances from lemon grass using co-solvent systems consisting of methanol or ethyl acetate and the ionic liquids 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl) or 1-ethyl-3-methylimidazolium methylphosphonate ([C_2mim][(MeO)(H)PO_2]) is described.

Keywords: Lemongrass, Ionic liquid, Extraction, Geranial, Neral, Geraniol.

INTRODUCTION

Lemon grass (*Cymbopogon citratus*) is an aromatic perennial plant that belongs to the Poaceae family. It is known as an herb and spice due to its lemon-like odor. In addition, the essential oils extracted from lemon grass are used in various foods, drinks, perfumes and cosmetics [1,2]. Thus, lemon grass is an important source of essential oils for the worldwide flavour and fragrance industries [3]. The essential oils obtained from lemon grass contain a complex mixture of monoterpenoids with geranial, neral and geraniol (Fig. 1) representing the most abundant components. The mixture of geranial [(*E*)-3,7-dimethyl-2,6-octadienal] and neral [(*Z*)-3,7-dimethyl-2,6-octadienal] is known as citral, which has a lemon-like scent. On the other hand, geraniol [(*E*)-3,7-dimethyl-2,6-octadien-1-ol] has a rose-like fragrance and is commonly used in perfumes [2]. Lemon grass is also recognized to have potential medicinal and biomedical applications. For example, it is used for the treatment of digestive disorders, fevers and rheumatism [3]. Moreover, the essential oils isolated from lemon grass exhibit antimicrobial activity [4], antifungal activity [5] and a hypocholesterolemic effect [3]. Consequently, they have received significant attention not only for their fragrances, but also their potential medicinal uses.

Presently, the most widely accepted method for the industrial extraction of essential oils is steam distillation. However, the extraction efficiency is not always satisfactory. We have therefore considered the use of ionic liquids for the effective extraction of essential oils as an alternative method of steam distillation. Ionic liquids are salts with melting points below 100 °C. Compared to conventional and typical organic solvents, they have various advantages, such as low volatility, low flammability, high thermal stability, excellent solubility for organic compounds and excellent ability to dissolve cellulose [6,7].

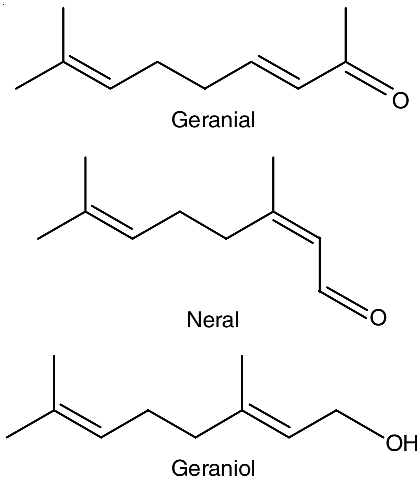

Fig. 1. Structures of geranial, neral and geraniol

The extraction of natural products using ionic liquids has attracted attention due to the growing interest in the development of new techniques with reduced environmental impacts [8]. In particular, 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl) [9] and 1-ethyl-3-methylimidazolium methylphosphonate ([C_2mim][(MeO)(H)PO_2]) [10] shown in

Fig. 2, are ionic liquids that dissolve cellulose, which is one of the main constituents of plant cell walls. While [C_4mim]Cl dissolves 10 wt % cellulose at 100 °C [9], [C_2mim][(MeO)(H)PO_2] dissolves 10 wt % cellulose at 45 °C [10]. We previously reported that nonvolatile natural products such as shikimic acid and bilobalide can be effectively extracted from *Ginkgo biloba* leaves using [C_4mim]Cl [11,12]. Ionic liquids have also been used for the extraction of essential oils from orange peels [13] and rosemary [14]. Thus, the efficient extraction of volatile natural products from plant leaves using ionic liquids has been considered to be possible [15]. Herein, we report the extraction of essential oils from lemon grass using [C_4mim]Cl and [C_2mim][(MeO)(H)PO_2] as major cellulose-dissolving ionic liquids as co-solvent with organic solvents such as methanol or ethyl acetate.

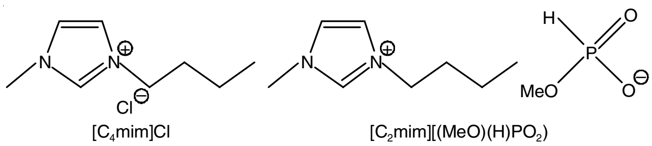

Fig. 2. Structures of [C_4mim]Cl and [C_2mim][(MeO)(H)PO_2]

EXPERIMENTAL

The ionic liquids [C_4mim]Cl and [C_2mim][(MeO)(H)PO_2)] were purchased from Sigma-Aldrich (St. Louis, MO) and Kanto Chemical Co., Ltd (Tokyo, Japan), respectively and used without further purification. Citral (> 93.0 %), geraniol (> 96.0 %), geranial (96 %) and methyl decanoate (> 99.5 %) were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Air-dried lemon grass leaves were purchased from Takeo Sodachi Happy Farmers (Saga, Japan) and stored at -30 °C before use. GC-MS analyses were performed using a Shimadzu GC-17A, GCMS QP5050A. The analytical conditions were as follows; column: DB-WAX capillary column (30 m in length, 0.25 mm i.d., 0.25 μm film thickness); temperature: 45 to 220 °C and held for 10 min; carrier gas: helium; flow rate: 1.7 mL/min; injection volume, 2.0 μL; solvents, MeOH and EtOAc. The retention times for methyl decanoate, neral, geranial and geraniol were approximately 9.7, 10.5, 10.9 and 11.8 min, respectively.

Extraction of geranial, neral and geraniol using organic solvents: The green leaves of lemon grass (3 g) were crushed using a mixer. The crushed leaves and solvent (MeOH or EtOAc; 16 g) were placed in a flask fitted with a reflux condenser and heated at reflux using a SynFlex (RCH-20L) temperature-controlled bath for 1 h. The mixture was then cooled to room temperature and filtered using a glass filter covered with Celite 545 to afford a solution (about 200 mL) containing the extract. 2 mL of mixture of EtOAc (10 mL) containing essential oil and methyl decanoate (2.5 mg) in EtOAc (10 mL) was injected into GC-MS.

Steam distillation: The green leaves of lemon grass (100 g) were subjected to steam distillation for 1 h using a steam distillation apparatus. The collected essential oil (about 500 mg) was then cooled to room temperature. 2 mL of mixture of essential oil (2.5 mg) in EtOAc (10 mL) and methyl decanoate (2.5 mg) in EtOAc (10 mL) was injected into GC-MS.

Extraction of geranial, neral and geraniol using [C_4mim]Cl or [C_2mim][(MeO)(H)PO_2]: The green leaves of lemon grass (3.0 or 1.5 g) were crushed using a mixer. The crushed leaves were added to [C_4mim]Cl (16 g) or [C_2mim[(MeO)(H)PO_2] (8 g) in a flask placed in a SynFlex (RCH-20L) temperature-controlled bath and stirred at 150 °C or 100 °C, respectively, for 1 h. After addition of H_2O (32 g or 16 g), the solution was stirred further for 30 min and then filtered using a glass filter covered with Celite 545. A solution of the extract was then obtained by dissolving the essential oils in EtOAc (100 mL or 50 mL), stirring for 30 min and then decanting the organic layer. Total volume of organic layer was about 300 mL. 2 mL in mixture of EtOAc (10 mL) containing essential oil and methyl decanoate (2.5 mg) in EtOAc (10 mL) was injected into GC-MS.

Extraction of geranial, neral and geraniol using [C_4mim]Cl/MeOH or [C_2mim][(MeO)(H)PO_2]/MeOH: The green leaves of lemon grass (3.0 g or 1.5 g) were crushed using a mixer. The crushed leaves were then added to [C_4mim]Cl/MeOH (3:1 w/w, 1:1 w/w) (16 g) or [C_4mim]Cl/EtOAc (3:1 w/w, 1:1 w/w) (16 g) or [C_2mim[(MeO)(H)PO_2]/MeOH (3:1 w/w, 1:1 w/w) (8 g), respectively, in a flask placed in a SynFlex (RCH-20L) temperature-controlled bath at 100 °C for 1 h. After addition of H_2O (32 g or 16 g), the solution was stirred further for 30 min and then filtered using a glass filter covered with Celite 545. A solution of the extract was then obtained by dissolving the essential oils in EtOAc (100 mL or 50 mL), stirring for 30 min and then decanting the organic layer. Total volume of organic layer was about 300 mL. 2 mL of mixture of EtOAc (10 mL) containing essential oil and methyl decanoate (2.5 mg) in EtOAc (10 mL) was injected into GC-MS.

Quantitative analysis of geranial, neral and geraniol using calibration curves: The obtained expressions for the calibration curves and correlation coefficients for each sample in EtOAc are as follows, where *x* and *y* represent the concentration and peak area, respectively:

$$\text{Geranial: } y = 0.94932\,x - 0.0143 \quad (R^2 = 0.9998)$$

$$\text{Neral: } y = 0.9570\,x - 0.00867 \quad (R^2 = 0.9996)$$

$$\text{Geraniol: } y = 0.9643\,x - 0.0128 \quad (R^2 = 0.9999)$$

RESULTS AND DISCUSSION

Extraction of the essential oils from lemon grass using organic solvents was first performed as control experiments (Fig. 3). The lemon grass was crushed by a mixer and refluxed with organic solvents MeOH or EtOAc for 1 h to obtain the rough extract, which was collected *via* filtration. Steam distillation of lemon grass on the laboratory scale was also performed as another control experiment. The quantities of geranial, neral and geraniol were determined *via* gas chromatography-mass spectrometry (GC-MS) analysis using calibration curves with methyl decanoate as the internal standard [2,16].

The extraction protocols using the cellulose-dissolving ionic liquids [C_4mim]Cl and [C_2mim][(MeO)(H)PO_2] are summarized in Fig. 3. When using [C_4mim]Cl, crushed lemon grass and [C_4mim]Cl or [C_4mim]Cl/MeOH (3:1 w/w, 1:1 w/w) or [C_4mim]Cl/EtOAc (3:1 w/w, 1:1 w/w) were placed in a flask

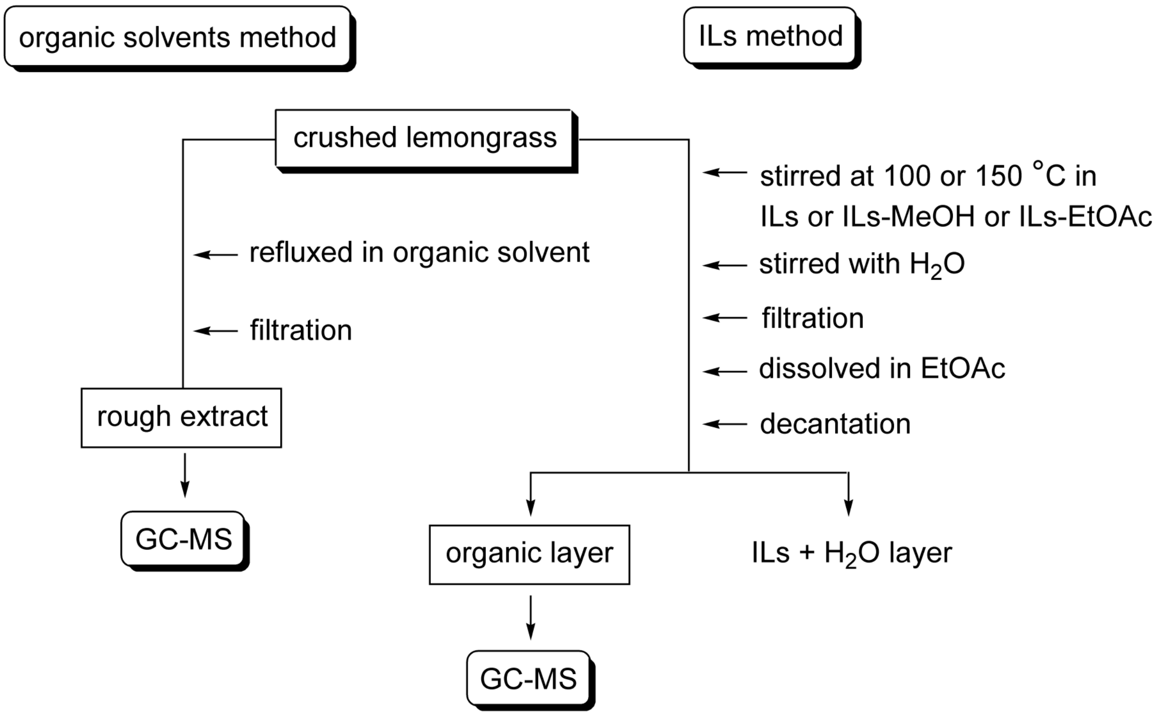

Fig. 3. Protocols for the extraction of essential oils from lemon grass using organic solvents and ionic liquids (ILs)

and heated for 1 h at 150 °C or 100 °C or 100 °C, respectively. When using [C_2mim][(MeO)(H)PO_2], crushed lemon grass and [C_2mim][(MeO)(H)PO_2] or [C_2mim][(MeO)(H)PO_2]/MeOH (3:1 w/w, 1:1 w/w) were placed and heated in a flask for 1 h at 100 °C. After heating, H_2O was added to the solutions to reduce the viscosities of the mixtures because ionic liquids have high viscosities. The mixed solutions were filtered and then diluted with EtOAc. The solutions were then stirred for 30 min and the essential oils in solution were decanted from the aqueous layer because both [C_4mim]Cl and [C_2mim][(MeO)(H)PO_2] do not dissolve in EtOAc. As with the substances isolated using organic solvents, the quantities of the essential oils extracted from lemon grass using the ionic liquids were determined *via* GC-MS analysis using calibration curves with methyl decanoate as the internal standard.

The extraction yields were calculated as the quantity of each essential oils over the original weight of the lemon grass leaves (Table-1). Comparing to MeOH and EtOAc (entries 1 and 2), extraction with EtOAc gave the better yields for geranial (0.613 %) and neral (0.219 %), while the yield for geraniol with MeOH was slightly better (0.0578 %). As expected, steam distillation afforded lower extraction yields (0.242 % for geranial, 0.139 % for neral and 0.0196 % for geraniol, respectively) comparing to extraction with organic solvents (entry 3). Use of ionic liquids only for the extraction afforded the relatively lower yields (entries 4 and 9). Among the use of co-solvents of [C_4mim]Cl or [C_2mim][(MeO)(H)PO_2] with MeOH or EtOAc (entries 5-8, 10-11), extraction for geranial with [C_4mim]Cl/EtOAc (1:1 w/w) gave the best yield (0.414 %). In the case of neral, [C_2mim][(MeO)(H)PO_2]/MeOH (1:1 w/w) afforded the best extraction yield (0.104 %). Although the extraction yields for geranial and neral using the ionic liquids were not greater than those obtained with EtOAc, the yields for geraniol using both [C_4mim]Cl and [C_2mim][(MeO)(H)PO_2]

TABLE-1
EXTRACTION YIELDS FOR GERANIAL, NERAL AND GERANIOL FROM LEMON GRASS USING ORGANIC SOLVENTS AND IONIC LIQUIDS

Entry	Solvents or method	Extraction yields (%)[a]		
		Geranial	Neral	Geraniol
1	MeOH	0.2680 ± 0.018	0.1320 ± 0.010	0.0578 ± 0.0055
2	EtOAc	0.6130 ± 0.031	0.2190 ± 0.012	0.0538 ± 0.0037
3	steam distillation	0.2420 ± 0.029	0.1390 ± 0.0043	0.0196 ± 0.0025
4	[C_4mim]Cl	0.0951 ± 0.0060	0.0534 ± 0.0041	0.0886 ± 0.0046
5	[C_4mim]Cl/MeOH (3:1 w/w)	0.2440 ± 0.0094	0.1030 ± 0.022	0.0983 ± 0.00024
6	[C_4mim]Cl/MeOH (1:1 w/w)	0.1720 ± 0.00051	0.0912 ± 0.00013	0.0954 ± 0.0027
7	[C_4mim]Cl/EtOAc (3:1 w/w)	0.1490 ± 0.0040	0.0799 ± 0.000078	0.0799 ± 0.0000078
8	[C_4mim]Cl/EtOAc (1:1 w/w)	0.4140 ± 0.017	0.0765 ± 0.00080	0.0764 ± 0.00080
9	[C_2mim][(MeO)(H)PO_2]	0.1400 ± 0.0026	0.0771 ± 0.0043	0.0869 ± 0.0014
10	[C_2mim][(MeO)(H)PO_2]/MeOH (3:1 w/w)	0.1610 ± 0.013	0.0867 ± 0.0065	0.0986 ± 0.0034
11	[C_2mim][(MeO)(H)PO_2]/MeOH (1:1 w/w)	0.1980 ± 0.0072	0.1040 ± 0.0029	0.1030 ± 0.0047

[a]Each experiment was run at least three times and the quantities are reported as average ± the standard deviation.

were much higher than those obtained when using MeOH. In particular, extraction yields with [C_4mim]Cl/MeOH (3:1 w/w) and [C_2mim][(MeO)(H)PO_2]/MeOH (1:1 w/w) were 0.0983 % and 0.103 %, respectively and thus gave 1.7 to 1.8 times as much geraniol as extracted with MeOH alone (0.0578 %).

It should be noted that the use of pure ionic liquids for the extraction of essential oils was difficult probably due to their nature of high viscosity. In the present study, the use of MeOH or EtOAc, which has a lower viscosity, as a co-solvent with the ionic liquids, promoted the stirring efficiency and thus contributed to the improved extraction yields for the essential oils. In addition, because the polarity of geraniol is much higher than that of geranial and neral, the use of ionic liquids with higher polarity was likely to contribute to the higher extraction yields observed for geraniol.

Since lemon grass is a family of Poaceae, major compositions in plant cell of lemon grass are known as not only hemicellulose but also lignin, which is a phenolic polymer [17]. Thus, use of 1-hexyl-3-methylimidazolium trifluoromethane sulfonate ([C_6mim][CF_3SO_3]), which is able to dissolve lignin [18] was attempted to extract essential oils from lemon grass. However, the extraction yields were quite low and even worse (data not shown). Use of other ionic liquids such as 1-ally-3-methylimidazolium formate ([Amim]HCOO) [19] and tetrabutylphosphonium hydroxide [10] was unsuccessful for the extraction in this study. Further investigation for efficient extraction of essential oils from lemon grass would be considered.

Conclusion

In summary, a new method has been developed for the extraction of essential oils from lemon grass using the ionic liquids [C_4mim]Cl and [C_2mim][(MeO)(H)PO_2)] with MeOH or EtOAc as extracting co-solvents. While the extraction yields for geranial and neral were not improved above that for pure EtOAc, the yield for geraniol was increased 1.7 to 1.8 times that obtained when using pure MeOH when mixtures of the ionic liquids and MeOH were employed. Further consideration for efficient extraction of essential oils from lemon grass and its related plant is currently underway in our laboratory.

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