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Convenient TLC-based Identification Test for the Crude Drug ''Pogostemoni Herba''

Yoshiaki AMAKURA,*[,a] Morio YOSHIMURA,[a] Chika MOURI,[b] Masayuki MIKAGE,[b] Nobuo KAWAHARA,[c] Yukihiro GODA,[c] and Takashi YOSHIDA[a]

[a]*College of Pharmaceutical Sciences, Matsuyama University, 4–2 Bunkyo-cho, Matsuyama City 790–8578, Japan, and* [b]*Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa City 920–1192, Japan, and* [c]*Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of Health Sciences, 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan*

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TLC and HPLC were used to identify possible chemical markers for evaluating the quality of the crude drug ''Pogostemoni herba'' (aerial part of *Pogostemon cablin*), which is a component of Kampo medicines. In addition to the reported patchouli alcohol and 2-hydroxy-6-methyl-3-(4-methylpentanoyl)-4-pyrone, three phenylethanoids were isolated from this plant material for the first time: acteoside, isoacteoside, and crenatoside. The usefulness of these compounds as indicators of the crude commercial drug under various TLC conditions was examined, and patchouli alcohol was found to give a definite spot with a reproducible *R*f value. Therefore, we propose TLC of the methanol (MeOH) extract using patchouli alcohol as a marker as a convenient method for identifying the crude drug Pogostemoni herba.

Key words——*Pogostemon cablin*; Pogostemoni herba; thin-layer chromatography; patchouli alcohol

INTRODUCTION

Pogostemon cablin Bentham (Labiatae) grows in Southeast Asia, China, and India, and its dried aerial part (Pogostemoni herba) is used as a crude drug called ''*Kakko*'' in Japan[1] for gastrointestinal disorders such as indigestion, vomiting, and diarrhea. ''*Kakko*'' is also an important component of Kampo formulas such as ''*Kakkoshokisan*.'' The crude drug is reported to contain terpenes,[2–4] flavones,[5,6] chalcones,[7] and alkaloids,[8] and is rich in volatile patchouli oil,[9–14] which is used widely in the perfume industry.

In traditional Chinese medicine, ''*Kakko*'' (or *Dokakko* or *Senkakko*) is derived from the leaves and stems of *Agastache rugosa* O. Kuntze (Kawamidori and Haikousou in Japan), which belongs to the same family, and the aerial part of *P. cablin* is called ''*Kokakko*.''[15,16] Although the Chinese *Kakko* derived from *A. rugosa* is currently rare in Japanese markets, a reliable quality standard for the crude drug Pogostemoni herba is necessary to avoid confusing its origin.

A method of identifying the crude drug Pogostemoni herba has already been described,[1] but it is a qualitative method that uses visualization with 2,4-dinitrophenylhydrazine reagent for carbonyl compounds in the distillate after steam distillation. Therefore, a simple, rapid method to detect marker compounds, such as a TLC method, is required. We describe a TLC-based method for identifying Pogostemoni herba as part of our studies on evaluating the quality of crude drugs.

*e-mail: amakura@cc.matsuyama-u.ac.jp

MATERIALS AND METHODS

General UV spectra were recorded on a Shimadzu UVmini-1240. Electrospray ionization (ESI) MS and high-resolution (HR) ESI-MS were performed using a micrOTOF-Q (Bruker Daltonics, USA) mass spectrometer with acetonitrile as the solvent. HR-EI-MS was performed on a JEOL JMS-700 instrument (JEOL, Tokyo, Japan). ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian INOVA AS600 (600 MHz for ^{1}H and 150 MHz for ^{13}C; Varian, USA), and the chemical shifts are given in parts per million relative to that of the solvent [chloroform-*d* (δ_H 7.26; δ_C 77.0) or methanol-d_4 (δ_H 3.30; δ_C 49.0)] on a tetramethylsilane scale. The standard pulse sequences programmed for the INOVA AS600 were used for each two-dimensional (2D) measurement (COSY, HSQC, and HMBC). J_{CH} was set at 8 Hz for the HMBC measurement. Reverse-phase HPLC conditions were: column, L-column ODS (5 μm, 150×2.1 mm i.d.; Chemicals Evaluation and Research Institute, Tokyo, Japan); mobile phase, solvent A 3% acetic acid and solvent B acetonitrile (0

−30 min, 0–50% B in A; 30–35 min, 50–85% B in A; 35–40 min, 85–85% B in A); injection volume, 10 μl; column temperature, 40℃; flow rate, 0.3 ml/min; and detection, 200–400 nm. Preparative HPLC conditions were: column, L-column ODS (5 μm, 250×10 mm i.d.; Chemicals Evaluation and Research Institute); mobile phase, solvent A 3% acetic acid and solvent B acetonitrile (0–30 min, 0–50% B in A; 30–35 min, 50–85% B in A; 35–40 min, 85–85% B in A); column temperature, 40℃; flow rate, 2.0 ml/min; and detection, 200–400 nm. Column chromatography was conducted on a Diaion HP-20, MCI GEL CHP-20P (75–150 μm; Mitsubishi Chemical Industries, Tokyo, Japan), Toyopearl HW-40 (fine grade; Tosoh, Tokyo, Japan), and silica gel 60 (Nacalai Tesque, Kyoto, Japan). TLC was carried out on silica gel 60 F_{254} plates (Merck, Germany). About 5 μl of the solution was injected, and the spots were visualized by heating the plates after spraying them with vanillin-sulfuric acid test solution[17] or by placing them under a UV lamp.

Materials Commercial Pogostemoni herba (*Kakko*) samples (A-E) were obtained from Uchida Wakanyaku (lot nos. 262314 and 263111, Tokyo, Japan), Tochimoto Tenkaido (lot nos. 025206001 and 025206002, Osaka, Japan), and Matsuura Yakugyo (lot no. 826084, Aichi, Japan) in 2006. The aerial parts of *A. rugosa* O. Kuntze were collected in September 1995 in Okayama, Japan, and in March 2008 in the medicinal plant garden of Kanazawa University, Ishikawa, Japan. A voucher specimen (MU-L-51) was deposited in the Herbarium of the College of Pharmaceutical Sciences, Matsuyama University, Ehime, Japan. All other chemicals were of analytical reagent grade.

Isolation Pogostemoni herba (400 g) was soaked in methanol (MeOH; 9 l) for 24 h at ambient temperature. The filtrate was concentrated to give an MeOH extract (40 g). The residue was further homogenized in 70% aqueous acetone (6 l), and the concentrated solution (1 l) was extracted with ethyl acetate (EtOAc; 6 l) to give EtOAc (6.0 g) and water (13.8 g) extracts. The MeOH extract was partitioned between *n*-hexane and water to give an *n*-hexane extract (11.9 g). A portion (1 g) of the *n*-hexane extract was dissolved in acetone and subjected to preparative TLC to give patchouli alcohol (**1**) (30 mg). Another portion (1 g) of the *n*-hexane extract was purified on column chromatography over silica gel 60 (50×2.0 cm i.d.) with *n*-hexane/acetone (9 : 1 →8 : 2) to give fraction A. Fraction A was purified using preparative TLC [*n*-hexane/acetone (9 : 1)] to give 2-hydroxy-6-methyl-3-(4-methylpentanoyl)-4-pyrone (**2**) (15 mg). A portion (1.0 g) of the EtOAc extract was subjected to column chromatography on MCI GEL CHP-20P (30×1.1 cm i.d.) with aqueous MeOH and then purified using preparative HPLC to yield acteoside (**3**) (30 mg), isoacteoside (**4**) (3.0 mg), and crenatoside (**5**) (2.0 mg).

RESULTS AND DISCUSSION

To characterize marker compounds for a TLC-based identification test of Pogostemoni herba, the optimal TLC conditions, including solvent and detection method, were examined for a sample solution prepared by shaking the crude drug in MeOH at ambient temperature. The TLC (SiO_2) chromatogram developed with *n*-hexane/acetone (9 : 1) was found to give two clear, well-separated spots with *R*f values of approximately 0.7 (spot 1) and 0.4 (spot 2). Spot 1 was visualized as purplish red by spraying with vanillin-sulfuric acid test solution followed by heating on a hot plate, and spot 2 was observed under UV light (254 nm). The constituent giving spot 1 was identified as patchouli alcohol (**1**)[2,3,18] based on its isolation using preparative TLC under the same conditions. The UV-sensitive compound (spot 2) was identified as 2-hydroxy-6-methyl-3-(4-methylpentanoyl)-4-pyrone (**2**).[19] On chromatographic separation of **2** over silica gel with HPLC monitoring, three phenylethanoid derivatives were also obtained: acteoside (**3**), isoacteoside (**4**),[20,21] and crenatoside (**5**)[22] (Fig. 1). All of the compounds were identified by spectral comparisons with reported data. Compounds **3**, **4**, and **5** were isolated from this plant material for the first time.

To determine the best marker among the constituents, TLC was performed for MeOH extracts obtained from five commercial samples of Pogostemoni herba (samples A-E) from Japanese markets. Compound **1** was detected as a clear spot with reproducible *R*f values in all samples (Fig. 2), whereas **2** was barely observed in some of the samples, probably because of low content (Fig. 3a). To confirm this, the MeOH extracts of samples A-E were analyzed using HPLC with a photodiode-array detector. Although all of the samples indicated the presence of **2** and **3**, the content of the former was clearly low in samples

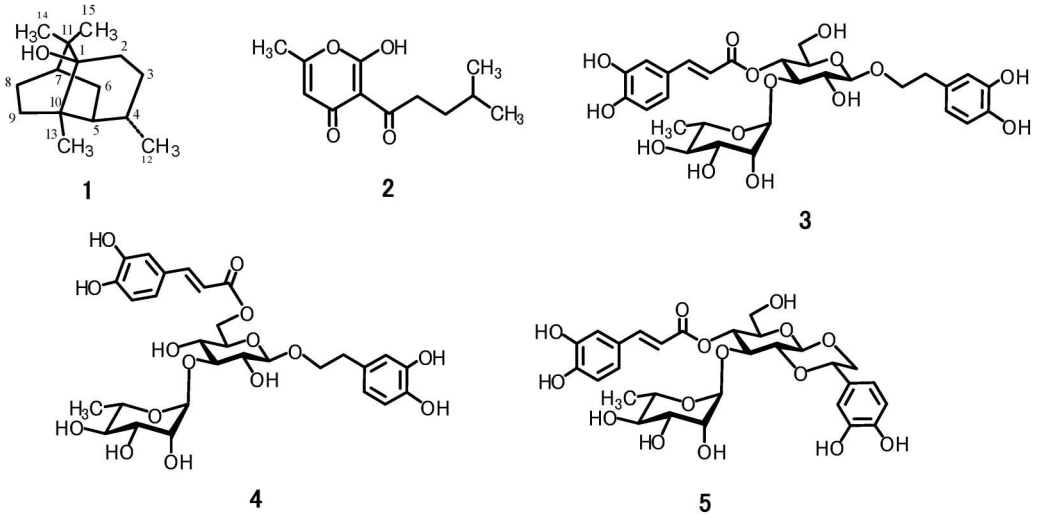

Fig. 1. Structures of Compounds **1**–**5** Isolated from Pogostemoni Herba

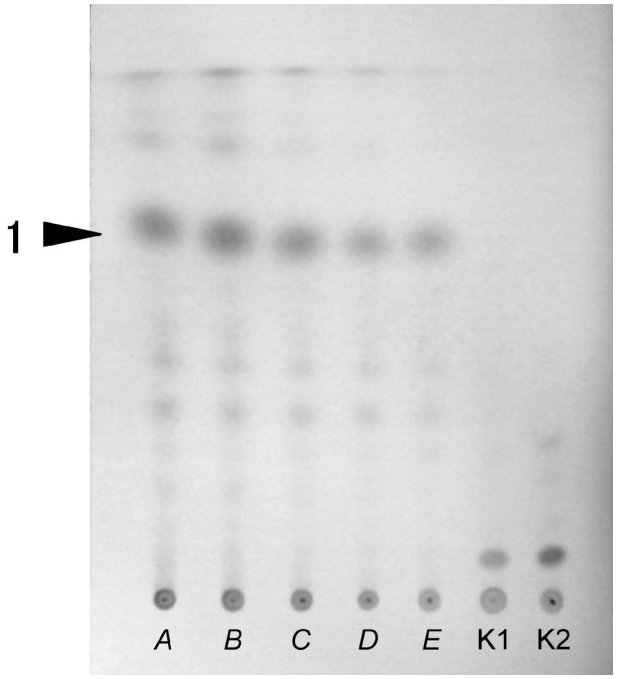

Fig. 2. TLC Profiles of MeOH Extracts (Samples A-E) of Commercial Pogostemoni Herba and MeOH Extracts of *A. rugosa* (K1 and K2)

1: Patchouli alcohol, Solvent: *n*-hexane-acetone (9 : 1), Detection: spraying the plate with vanillin-sulfuric acid test solution and heating.

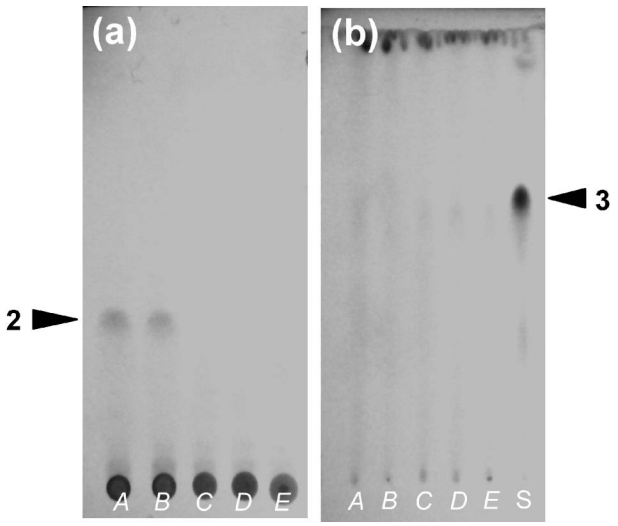

Fig. 3. TLC Profiles of MeOH Extracts (Samples A-E) of Commercial Pogostemoni Herba

(a) Solvent: *n*-hexane-acetone (9 : 1), Detection: UV lamp at 254 nm. (b) S: acteoside (**3**), Solvent : ethyl acetate : acetic acid : water (7 : 2 : 1), Detection: UV lamp at 254 nm.

C, D, and E (Fig. 4). Therefore compound **2** is not suitable as a marker for TLC, although it has potential as a diagnostic marker because it is a rare characteristic constituent of this plant and is easily detected under UV light without any reagent. Likewise, compound **3** is also not adequate as a TLC marker because its spot was not detected clearly in all samples prepared using simple MeOH extraction and UV irradiation (Fig. 3b) or spraying with the detection reagent, iron (III) chloride or 4-methoxybenzaldehyde-sulfuric acid.[23] These results were similar to those for drug extracts prepared under heat (60°C, 10 min). Therefore we deemed compound **1** the best marker for a TLC-based method for authenticating the crude drug.

To distinguish Japanese "*Kakko*" from the Chinese "*Kakko*" derived from *A. rugosa*, the MeOH extract of *A. rugosa* was subjected to TLC. Compared with the TLC profiles of samples A-E, the spot of **1** was not detected on the TLC plates of two

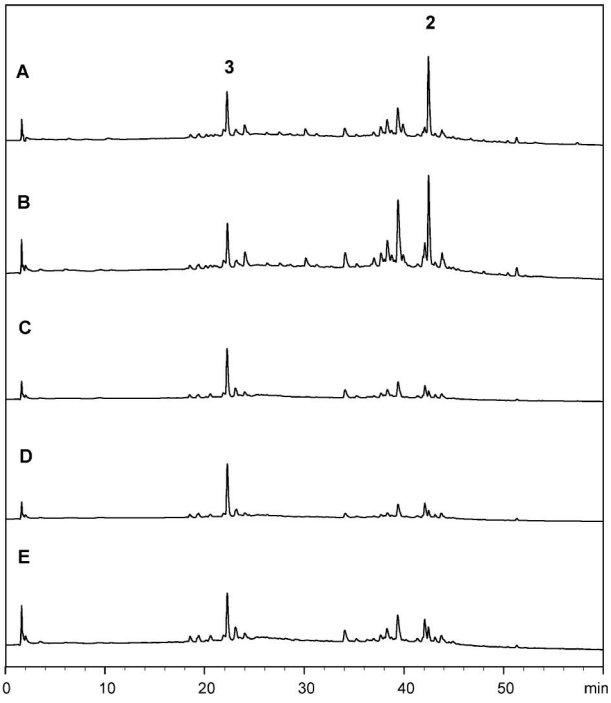

Fig. 4. Reversed-phase HPLC Profiles at 254 nm of MeOH Extracts (Samples A-E) of Commercial Pogostemoni Herba

A. rugosa extracts (K1 and K2; Fig. 2), allowing the differentiation of these crude drugs, although the Chinese "*Kakko*" itself was not examined in detail.

In conclusion, our proposed TLC identification test for Pogostemoni herba is as follows: the sample (0.5 g) is extracted by shaking with MeOH (5 ml) for 3 min at ambient temperature and then filtered. An aliquot (5 μl) of the filtrate is applied to a TLC plate and developed with a mixture of *n*-hexane/acetone (9 : 1) to a distance of 10 cm, and the plate is air-dried. The plate is sprayed thoroughly with vanillin-sulfuric acid test solution and heated to detect the purplish red spot of **1** with an *R*f value of approximately 0.6–0.7. This method consists of a simple MeOH extraction procedure and TLC using safe reagents [*n*-hexane/acetone (9 : 1)] without harmful benzene or halogenated solvents such as chloroform and dichloromethane and is comparable to the identification tests used for many crude drugs in the *Japanese Pharmacopoeia*, 15th edition.[17]

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