Original article

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AN APPLICATION OF TLC-IMAGE ANALYSIS FOR INVESTIGATION OF AQUEOUS EXTRACT OF COMMON CULINARY HERBAL COMBINATION, KAFFIR LIME LEAF, GALANGAL AND LEMON GRASS RHIZOMES

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ABSTRACT

Kaffir lime leaf, galangal, and lemon grass rhizomes are ones of the common kitchen herbs used as flavoring ingredients in famous traditional Thai cuisine, called TomYum. These herbs are also employed as herbal medicines for a wide range of applications. A number of studies were conducted to investigate phytochemical constituents of these household remedy, however, the gas chromatographic (GC) and high performance liquid chromatographic (HPLC) analyses of volatile oils or essential oils in each individual herbal extract prepared by low to partial polar organic solvents were mainly focused. Since combinations of these herbs are usually prepared by brewing with hot water, therefore, this study presented the use of TLC-image analysis method for visualization of all detectable constituents in the aqueous extract of the multi-ingredient preparation. Good chromatographic separations of various substance classes were achieved on silica gel 60 F_{254S} TLC plates with the use of several mobile phase compositions, multiple detections and TLC-image analysis software, Sorbfil TLC Videodensitometer, to create a chromatographic profile.

Keywords: TLC-image analysis, kaffir lime leaf, galangal rhizome, lemon grass rhizome, fingerprint

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Introduction

The use of herbs as food, food flavorings or specially as herbal remedies for prevention and treatment of disease has risen significantly during the past decade.[1] Some culinary herbs, valued for their aromatic or savory or medicinal qualities, can be used for multiple purposes. The leaf of Kaffir lime (*Cymbopogon citratus* (DC) Stapf., family Gramineae), rhizome of galangal (*Alpinia galanga* (L.) Willd., family Zingiberaceae), and rhizome of lemon grass (*Citrus hystrix* DC., family Rutaceae) are ones of the common kitchen herbs used as flavoring ingredients in famous traditional Thai cuisine, called Tom-yum.

These herbs are also employed as herbal medicines for a wide range of applications. For example, several therapeutic uses of lemon grass have been reported including antibacterial, antifungal, antispasmodic, anti-inflammatory, cardioprotective activity, *etc*.[2] The medicinal applications of galangal have been reported for the treatment of gastric diseases, gout and colic.[3,4] Antibacterial activity of kaffir lime leaf has also been reported.[5,6]

A number of studies were conducted to investigate phytochemical constituents of these household remedy. Essential oils are the major class of compounds being reported.[3-9] Additionally, a number of other classes of compounds such as flavonoids, alkaloids, phenols, tannins, saponins and anthraquinones were also described.[2-12]

Several analytical methods, based on the use of gas chromatography (GC) and high performance liquid chromatography (HPLC), have been reported for the study of chemical compositions in lemon grass[10-14], galangal[4,15] and kaffir lime leaf.[16,17] However, these previous studies mainly focused on the analysis of volatile oils or essential oils in each individual herbal extract prepared by low to partial polar organic solvents. Chromatographic profiles of aqueous extracts of the mixed ingredients have not been previously reported. Since combinations of these herbs are usually used in household cooking by brewing with hot water and some used as herbal tea product for health benefits, therefore, it is of interest to investigate all detectable constituents in the aqueous extract of the multi-ingredient preparation. One of the analytical approaches that is useful for the analysis of the complex herbal combinations is thin-layer chromatography.[18] Along with the use of an image analysis approach, the chromatographic profile represented as peak data could be obtained in this study.

Materials and methods

Plant materials

The dried herbal raw material, kaffir lime leaf, galangal rhizome and lemon grass rhizome, were obtained from Nakhon Pathom province, Thailand, during 2016, and identified by Associate Professor Uthai Sotanaphun. Voucher specimens (US-01 to US-03) are deposited at the Herbarium of the Department of Pharmacognosy, Silpakorn University, Thailand. Each was chopped into a small piece. The mixture of kaffir lime leaf, galangal and lemon grass at a ratio of 1:2:1 was extracted twice with 10 times by weight of water at 100°C for 2 hr and evaporated to dryness by rotary evaporator. An aqueous extract of each herb was also prepared separately as above. The dried aqueous extracts of the mixture and each herb were reconstituted in water at a concentration of 100 mg mL^{-1} and 30 mg mL^{-1}, respectively, for TLC analyses.

Chromatographic conditions

TLC analysis was performed on TLC silica gel 60 F_{254S} aluminium plates (12 cm x 10 cm with 0.25 mm thickness, Merck, Germany). Fifteen µL of each aqueous solution were applied as a 10 mm band onto a TLC plate using a TLC sampler Linomat 5 (Camag, Switzerland). The plate was developed to a distance of 10 cm in a TLC chamber. Four different mobile phases, ethyl acetate-formic acid-acetic acid-water (100:11:11:26, *v/v/v/v*), *n*-hexane-ethyl acetate-acetic acid (5:3:1, *v/v/v*) preconditioning with

HCl in the opposite trough of twin chamber, toluene-ethyl acetate (93:7, *v/v*) and toluene-acetone-formic acid (7:3:0.1, *v/v/v*) were used. Then, the plate was air-dried at room temperature. Each TLC plate was visualized under UV light at 254 nm, 366 nm, white light after the plate was derivatized with anisaldehyde reagent (0.5 mL *p*-anisaldehyde, 1 mL H_2SO_4 in 50 mL AcOH) and warmed for 5 min at 110 ^{o}C, and UV 366 nm after derivatization with anisaldehyde. All TLC images were recorded by using a CAMAG TLC visualizer. All chemicals and solvents used were analytical reagent grade.

For validation, **s**ample solutions of the aqueous extracts were found to be stable on TLC plates during analyses. Robustness of the TLC methods was studied by introducing small changes in the mobile phase compositions. The repeatability of TLC method was determined by performing each analysis in triplicate. Rf values for all major bands observed in each TLC analysis were recorded for robustness and repeatability evaluation. A reasonable acceptance criterion would be that Rf values of the same band should not vary more than 0.02 from plate to plate.[19]

TLC-image Analysis

For TLC-image analysis method, the color image of the plate was saved as a joint photographic experts group (JPEG) file. By using PhotoScape V3.6, a free photo editing software, the JPEG image was resized at a resolution of 100 pixels in height with preserved aspect ratio and adjusted to high auto level and high auto contrast for image analysis by Sorbfil TLC Videodensitometer software (Sorbpolymer, Russia). The evaluation of each track was processed by using Process Track command. A chromatogram was constructed on the deviation of track intensity from background intensity.

Results and discussion

In this study, TLC analyses of the aqueous extract of the culinary herbal combination were investigated and compared with those of each individual herb. Due to the large variability of chemical constituents, multiple TLC fingerprints representing various substance classes were obtained by using several mobile phase compositions and multiple detections.

Two common mobile phases, ethyl acetate-formic acid-acetic acid-water (100:11:11:26, *v/v/v/v*), and *n*-hexane-ethyl acetate-acetic acid (5:3:1, *v/v/v*) preconditioning with HCl in the opposite trough of twin chamber, were used for separation of partial polar to polar compounds on a silica gel plate. The fingerprints of nonpolar to low polar region were obtained by the use of mobile phases composed of toluene-ethyl acetate (93:7, *v/v*) and toluene-acetone-formic acid (7:3:0.1, *v/v/v*), respectively. Four different detections, UV 254 and 366 nm, white light after derivatization with *p*-anisaldehyde, and fluorescence under UV 366 nm after derivatization, were performed on the same plate. *p*-Anisaldehyde is a nonspecific chemical staining solution and commonly used as a universal visualization method for examining TLC. By generating multiple fingerprints, various substance classes in the aqueous extract of multi-ingredients could be obtained in different features, i.e., polar and nonpolar compounds with UV-absorbing properties and with a range of colors depending on chemical classes (Figure 1). For example, a presence of phenols, terpenes, sugars, and steroids could be seen in the extracts as violet, blue, red, grey or green bands after derivatization with *p*-anisaldehyde.

The fingerprints of all tracks were compared with respect to number, sequence, position and color of separated zones. Based on different chromatographic systems, multiple fingerprints of the aqueous extracts provided comprehensive information of the mixed ingredient. From side-by-side comparison between the fingerprint of each herb and that of the mixture, the presence of predominant bands in each track of the individual herb was also found in the fingerprint of the aqueous extract of the herbal mixture.

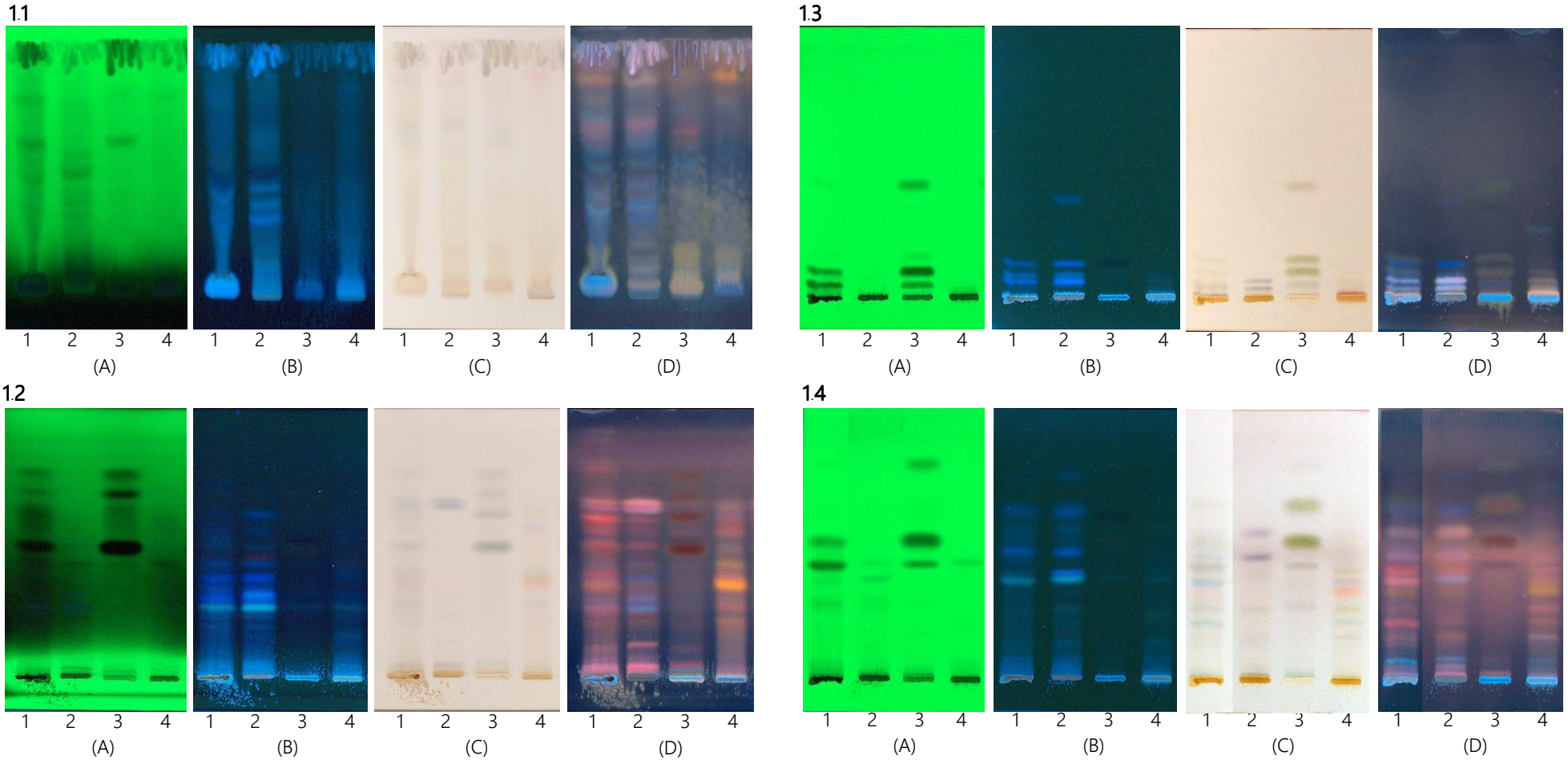
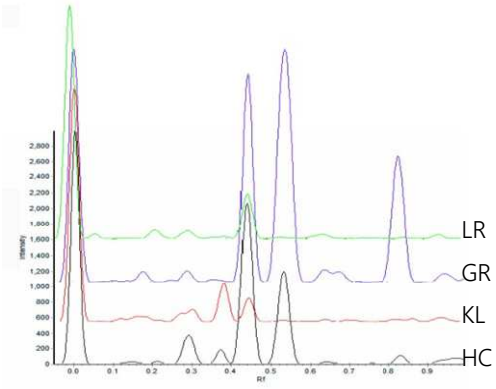


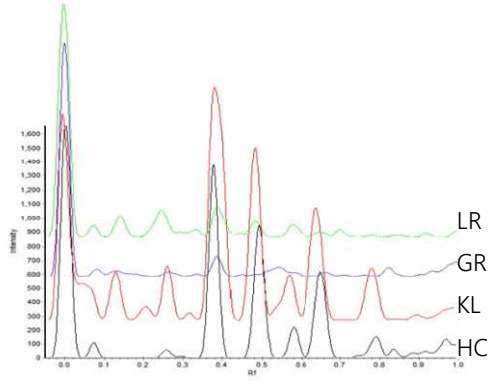
Figure 1 TLC fingerprints of aqueous extracts of the herbal combination and each individual herb using several mobile phase compositions and multiple detections. Track 1, aqueous extract of the herbal combination; tracks 2-4, aqueous extract of kaffir lime leaf, galangal rhizome, and lemon grass rhizome, respectively. **1.1**, Ethyl acetate-formic acid-acetic acid-water (100:11:11:26, *v/v/v/v*); **1.2**, *n*-hexane-ethylacetate-acetic acid (5:3:1, *v/v/v*), preconditioning with HCl in the opposite trough of twin chamber; **1.3**, toluene-ethyl acetate (93:7, *v/v*); and **1.4**, toluene-acetone-formic acid (7:3:0.1, *v/v/v*). (A), Detection under UV 254 nm; (B), under UV 366 nm; (C), under white light after derivatization with anisaldehyde; and (D), under UV 366 nm after derivatization with anisaldehyde. (For color figure, please refer to the web version of this article.)

For qualitative analysis, the robustness and repeatability of the method were validated during its development and found to be acceptable since the measured Rf values of the same bands obtained from each analysis having small changes in the mobile phase compositions and upon running each analysis in triplicate varied by less than 0.02. Furthermore, with a combination of TLC image analysis software, a chromatographic profile was obtained (Figure 2). The major peak owing to kaffir lime leaf, galangal and lemon grass rhizomes could be clearly identified in the aqueous extract of the culinary herb combination.

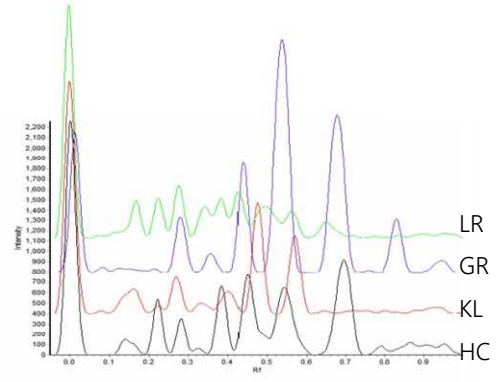
By generating multiple TLC-images using various mobile phases and detections, proper investigation of complex herbal products could be significantly improved especially when none of chemically defined markers are available. In a case where there were no specific markers used as decision criteria, the certainty of the identification could be best achieved by the use of images.[19] Together with more convenient and less expensive computer technology, fingerprints preserved in a form of electronic images could be established as an integral part of official documents for quality control in the small-scale herbal industries with limited resources.



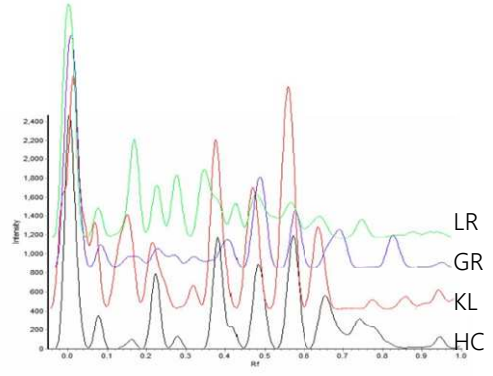
(A) UV 254 nm



(B) UV 366 nm



(C) white light after derivatization with anisaldehyde



(D) 366 nm after derivatization with anisaldehyde

Figure 2 Chromatographic profiles of Figure 1.4 of aqueous extracts of HC (herbal combination), KL (kaffir lime leaf), GR (galangal rhizome) and LR (lemon grass rhizome) under various detections (A-D).

Conclusion

An application of TLC-image analysis for examining the complexity of the herbal preparations in its entirety was demonstrated herein. With the possibility to obtain different features of TLC fingerprints as images or transformed into peak data (which could be useful for further quantitative determination) in a rapid, simple and economical manner, a TLC-image analysis could be considered as an alternative method for quality assessment of complex herbal material.

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