ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 26 (5) (2023): 187-193

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Thin Layer Chromatography Fingerprint Analysis of Tempuyung (*Sonchus arvensis* L.)

Sri Fauziah [a], Puput Melati [b], Ade Heri Mulyati [c], Mohamad Rafi [a,b], Eti Rohaeti [a,b,*]

[a] Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Jalan Tanjung Kampus IPB Dramaga, Bogor 16680, Indonesia
[b] Tropical Biopharmaca Research Center-Institute of Research and Community Service, IPB University, Jalan Taman Kencana No.3 Kampus IPB Taman Kencana, Bogor 16128, Indonesia
[c] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor 16143, Indonesia

* Corresponding author: etirohaeti@apps.ipb.ac.id

https://doi.org/10.14710/jksa.26.5.187-193

Article Info

Article history:

Received: 27th January 2023
Revised: 22nd June 2023
Accepted: 04th July 2023
Online: 31st July 2023

Keywords:
Tempuyung; quality control; TLC

Abstract

Thin layer chromatography (TLC) fingerprint profile analysis can be used for quality control of herbal medicinal raw materials through identification, authentication, and discrimination. This study aims to develop a fingerprint analysis method for *tempuyung* TLC (*Sonchus arvensis* L.), which is then used for quality control. Tempuyung was extracted with methanol using ultrasonication which was then analyzed using the developed fingerprint TLC method. The optimum mobile phase used to separate compounds from tempuyung was a composition of chloroform: ethyl acetate: dichloromethane: formic acid (7.5:2:0.5:0.1) and produced eleven bands. The mobile phase composition produced good separation and had a typical luteolin band with an R_f value of 0.22, detected under UV 366 nm and derivatized with 10% sulfuric acid reagent. This method was applied to tempuyung from three locations, including Malang, Solo, and Yogyakarta, whose fingerprint patterns did not differ significantly. The fingerprint method has been validated and met the acceptance requirements for quality control of tempuyung.

1. Introduction

Tempuyung (*Sonchus arvensis* L.) plant belongs to the Asteraceae family and is a non-nitrophilic species that can grow in nutrient-poor soil. Tempuyung is widespread in several continents, including Asia, Europe, and Africa. The distribution of tempuyung in Indonesia is found widely in Sumatra, Java, Bali, Papua, Kalimantan, and Sulawesi [1]. The content of tempuyung compounds in these areas is different [2] due to several factors, such as growing location [3], soil nutrients, temperature, and weather. Tempuyung, which contains a large number of bioactive compounds [4], has been widely used as a raw material for herbal medicine due to its wide range of pharmacological properties, including antioxidant [5], anti-inflammatory [6], diuretic, antibacterial [7], and anticancer [1]. These bioactive compounds come from alkaloids, flavonoids, saponins, triterpenes, and steroids [8]. Examples of flavonoid compounds include orientin, quercetin, catechins, rutin, myricetin, luteolin, apigenin, and kaempferol [9]. Luteolin is one of the flavonoid molecules that is a distinctive component of tempuyung [10]. Among the many benefits of tempuyung, Xia and Liang [11] found that the plant can be an insecticide. According to Seal [12], Indians also reportedly eat tempuyung leaves as a salad.

Quality control must be developed as tempuyung is increasingly used in pharmaceutical products, particularly when providing premium raw materials. This quality control is necessary for ensuring the product's suitability for the purpose, consistency, efficacy, and safety [13] and for guaranteeing the pharmacological activity of the products [14]. Additionally, quality control is practiced to prevent the counterfeiting of raw materials using plants that are morphologically or closely related to one another. The absence of quality control can cause inconsistent efficacy and safety, harming human health. One of the analytical techniques that can be used to control tempuyung quality is the fingerprint analysis

approach using thin layer chromatography (TLC) [15]. This fingerprint profile analysis provides information about the compounds contained in the sample. This analysis can be utilized to identify, authenticate, and discriminate herbal medicinal raw materials. This analysis provides information on the broad and detailed chemical compound profile that can be used as a reference for quality control [16].

Thin layer chromatography (TLC) is a simple chromatographic analysis technique widely used for plant fingerprint analysis. This method is widely utilized as a separation method in quality control because of its simplicity of usage, accessibility, and affordability [17]. TLC can be used for qualitative analysis by examining the separated fingerprint profile based on the position, color, quantity, intensity, and R_f (retardation factor) of the resulting bands. The fingerprint TLC method has been widely used in several studies, such as the analysis of metabolite profiles of the rhizome of intersection mango (*Curcuma mango*) [18], differentiating simplicia powders of turmeric, bangle, *temulawak* [15], and Dutch teak leaves (*Guazuma ulmifolia*). Several previous related studies have not reported the fingerprint analysis of tempuyung plants using the TLC method.

2. Methodology

2.1. Materials and Instrument

Tempuyung plants (*Sonchus arvensi* L.) were collected from Malang, Solo, and Yogyakarta. Silica 60 F254 TLC plates, sulfuric acid, hexane, diethyl ether, dichloromethane, chloroform, ethyl acetate, and methanol were purchased from Merck (Darmstadt, Germany). Those chemicals were of analytical grade, excluding acetone, acetonitrile, and ethanol (Merck, Darmstadt, Germany).

The equipment used were ultrasonicator Branson 1510 (Branson, Danbury, USA), TLC semiautomatic applicator Camag Linomat 5 (CAMAG, Muttenz, Switzerland), WinCATS application (CAMAG, Muttenz, Switzerland), twin trough chromatography chamber (10 × 20 cm), flat-bottom chamber (20 × 20 cm) (CAMAG, Muttenz, Switzerland), and CAMAG Repostar 3 (CAMAG, Muttenz, Switzerland).

2.2. Procedures

2.2.1. Sample Extraction

The extract was obtained by extracting 1 gram of sample with 10 mL of methanol using an ultrasonicator for 30 minutes at room temperature. The extraction results were filtered (gravity filtration), put into another bottle, and then covered with aluminum foil.

2.2.2. Plate Preparation and Sample Application

TLC plates were cut into three different sizes: 13 × 10 cm, 10 × 10 cm, 4 × 10 cm, and 2 × 10 cm. The horizontal lines of about 1 cm each were drawn on plates from the bottom of the plate as the starting line and from the top as the finishing line. The plate was eluted to the finishing line using methanol solvent in a chamber saturated for 10 minutes. The plate was then dried in the oven at 100°C for 30 seconds and utilized for a sample application. Sample spotting was performed using a TLC applicator with a spotting speed of 80 nL/s, a sample volume of 10 µL and a standard of 5 µL, a bandwidth of 8 mm, and a spacing of 1.2 mm between spots.

2.2.3. Mobile Phase Selection

The sample extract was applied on the dried plate using the TLC applicator. The plate was then put into 10 mL of a single solvent in a chamber saturated for 10 minutes to elute until it reached the finishing line. After the elution, the plates were removed, dried at room temperature, and observed under UV lights (254 and 366 nm). The plates were derivatized using 10% sulfuric acid, dried in an oven at 100°C for 5 minutes, then observed under UV light at 366 nm. The solvents used were *n*-hexane, diethyl ether, chloroform, ethyl acetate, dichloromethane, acetone, acetonitrile, ethanol, and methanol. The best chromatogram profile obtained from a single mobile phase was then formulated to obtain the optimum mobile phase and compared with the luteolin standard.

2.2.4. Method Validation for the Best Mobile Phase Mixture

The best mobile phase obtained was validated with some parameters, including stability, specificity, precision, robustness of chamber, and development distance [16]. The stability of the analyte was determined by developing two-dimensional chromatography. In contrast, the analyte in the plate was determined by comparing the fingerprint pattern resulting from the difference in the delay time of the plate before and after the sample was applied. Specificity was done by comparing the samples with plants with similar morphology, namely *tapak liman*. Precision was performed by repeating the process thrice and on different days. Robustness was measured by comparing the separation results using twin-trough and flat-bottom chambers.

2.2.5. Application of TLC Separation Method

The developed TLC separation method was applied by measuring tempuyung plants from three locations with three repetitions for each analysis. The obtained fingerprint patterns were then processed using the ImageJ application program to determine the differences in intensity of the fingerprint patterns of the tempuyung in the three locations. This application can change the bands on the chromatogram into a densitogram, a graph of the relationship between the intensity and the R_f value.

The densitogram process was done by storing the results of documentation detected at UV 366 nm in .jpg format. Chromatogram analysis using ImageJ was started by marking the fingerprint pattern bands using Analyze-Gels-Select first lane on the Rectangular menu and selecting the next line for the next band if more than one band was processed. The Analyze-Gels-Plot lane menu was re-selected to display the densitogram matching the fingerprint pattern. The densitogram was then converted into a line graph with XY coordinates using the Analyze-Tools-Analyze line graph menu. Information in numeric

format could be obtained by pulling all the data on the line graph. The data was then processed using the Origin software to form further curves.

3. Results and Discussion

3.1. Best Mobile Phase

The ideal mobile phase was selected to obtain the optimal separation quality, which started with developing several solvents with various properties, ranging from non-polar to polar. This selection was done so that analytes with various polarity levels could be separated appropriately. It is necessary to pay attention to the resolution value and the number of bands, as both are good separability parameters for the TLC profile. The resolution value equal to one (=1) is the minimum value [16], and more than 1.5 is the best value. The results of developing a single solvent with various polarity indexes were then detected with UV lights at 254 and 366 nm to achieve maximum visualization of the TLC profile (Figures 1 & 2).

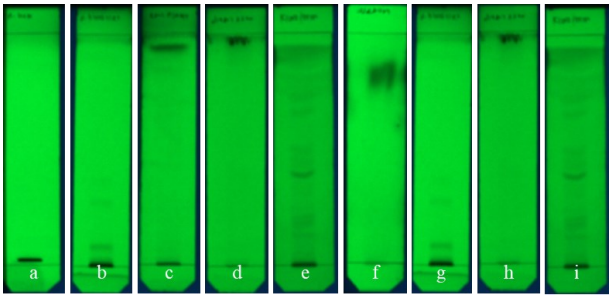

Figure 1. TLC profiles of tempuyung plants under UV 254 nm with single mobile phases of a) *n*-hexane, b) dichloromethane, c) ethyl acetate, d) diethyl ether, e) chloroform, f) acetone, g) acetonitrile, h) ethanol, and i) methanol

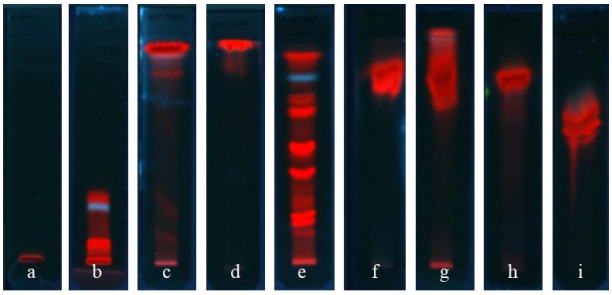

Figure 2. TLC profiles of tempuyung plants under UV 366 nm with single mobile phases of a) *n*-hexane, b) dichloromethane, c) ethyl acetate, d) diethyl ether, e) chloroform, f) acetone, g) acetonitrile, h) ethanol, and i) methanol

The TLC profile with the best visualization was obtained from UV detection at 366 nm. This can be seen from the appearance of the band stains produced on each plate, which are more clearly visible (Figure 2). Detection for subsequent experiments was carried out under 366 nm UV light. The single solvent that produces the best separation is obtained from the single solvent chloroform, ethyl acetate, and dichloromethane (Figure 2), where the three bands produce good separation resolution with the highest number of bands. These three solvents were then selected to be formulated with various comparisons to obtain optimal separation results. The composition of the mobile phase mixture used was chloroform: dichloromethane with a ratio of (A) 5:5, (B) 6:4, (C) 7:3, (D) 8:2, (E) 9:1, chloroform: ethyl acetate with a ratio of (F) 7:3, (G) 8:2, (H) 9:1, and chloroform: ethyl acetate:dichloromethane: formic acid with a ratio of (I) 6:3:2:0.1, (J) 6.5:2:1.5:0.1, (K) 7:2:1:0.1, (L) 7.5:2:0.5:0.1, (M) 8:1.5:0.5:0.1. The TLC profile resulting from the use of a mixture of solvents with various comparisons can be seen in Figure 3.

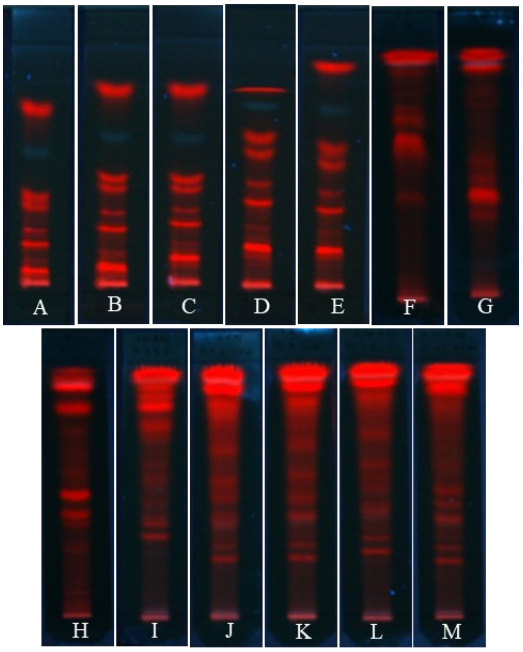

Figure 3. The number of mixed mobile phase bands

The separation between the bands, the number of bands, and the resolution resulting from the use of various mobile phase mixture compositions in each TLC profile differed from each other due to the difference in the degree of polarity of the mobile phase mixture for the separation of flavonoid compounds from tempuyung. The formic acid solvent was added to the mobile phase mixture code I to M to reduce the tailing effect on the band [10]. Data on the number of bands produced from the several mobile phase compositions can be seen in Figure 4.

The highest number of bands obtained from using a solvent composition with code L is 11 bands. Another parameter to determine the best mobile phase can be seen from the resolution value. The resolution value produced by the mixed mobile phase with code K is considered good because it exceeds 1.5. However, the number of bands produced is not optimal; thus, derivatization was performed to produce the best separation. The best mobile phase composition was obtained using an eluent mixture with code L, which produced the highest number of bands (11 bands) with a resolution value greater than 1.5. These results were obtained using 366 nm UV irradiation after derivatization (Figure 5). The calculation results for the Rf value and resolution for the mobile phase mixture K and L from the TLC profile can be seen in Table 1.

Table 1. Band resolution values of chloroform: ethyl acetate: dichloromethane: formic acid mobile phase with a ratio of 7:2:1:0.1 and 7.5:2:0.5:0.1 under UV 366 nm

Comparison of mobile phase compositions	Band	Band distance (cm)	Band width (cm)	R_f	Resolution
Chloroform: ethyl acetate: dichloromethane: formic acid (7:2:1:0.1)	1	0.08	0.1	0.01	-
	2	1.92	0.1	0.24	18.4
	3	2.23	0.1	0.29	4
	4	3.2	0.2	0.4	5.87
	5	3.76	0.2	0.47	2.8
	6	4.48	0.2	0.56	3.6
	7	5.52	0.2	0.69	5.2
	8	6.8	0.2	0.85	6.4
	9	7.12	0.2	0.89	1.6
	10	7.92	0.3	0.99	3.2
Chloroform: ethyl acetate: dichloromethane: formic acid (7.5:2:0.5:0.1)	1	0.16	0.1	0.02	-
	2	2.08	0.1	0.15	19.2
	3	2.56	0.1	0.2	4.8
	4	3.52	0.3	0.25	4.8
	5	4.16	0.2	0.3	4.8
	6	4.56	0.2	0.38	2
	7	4.96	0.2	0.41	2
	8	5.84	0.2	0.5	4.4
	9	6.96	0.1	0.53	7.47
	10	7.36	0.2	0.6	2.67
	11	7.92	0.3	0.68	2.24

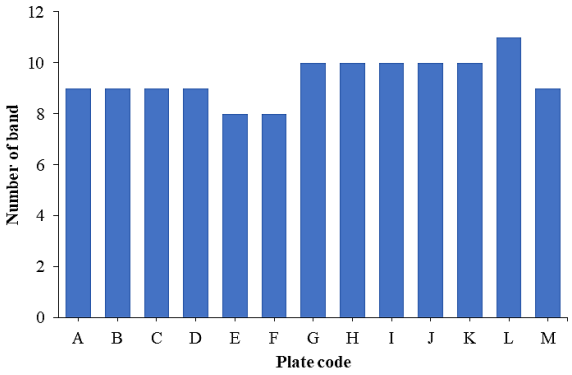

Figure 4. The number of mobile phase bands of a mixture of chloroform: dichloromethane (A) 5:5, (B) 6:4, (C) 7:3, (D) 8:2, (E) 9:1, chloroform: ethyl acetate (F) 7:3, (G) 8:2, (H) 9:1, and chloroform: ethyl acetate: dichloromethane: formic acid (I) 6:3:2:0,1, (J) 6.5:2:1.5:0.1, (K) 7:2:1:0.1, (L) 7.5:2:0.5:0.1, (M) 8:1.5:0.5:0.1

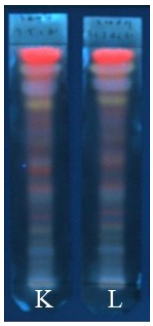

Figure 5. TLC profile of a mixture of chloroform: ethyl acetate : dichloromethane: formic acid mobile phase with a ratio of K) 7:2:1:0.1 and L) 7.5:2:0.5:0.1 derivatized results

The chromatogram of the separation results using the mobile phase code L produced a band of luteolin compounds with an R_f value of 0.20 (y). The R_f value is not significantly different from the standard luteolin R_f value of 0.21 (x) (Figure 6).

Figure 6. Comparison of standard bands of luteolin (x) with luteolin in a sample (y) under UV 366 nm

The standard luteolin band and the sample luteolin glow differently due to the difference in concentration. The standard concentration of luteolin was 200 ppm, whereas a previous study reported that the concentration of the luteolin compound in the tempuyung plant was 12.57 ppm [9]. Standard luteolin with a concentration of 200 ppm is used as a comparison so that the resulting band is clearer, making it easier to calculate the Rf value and resolution. Luteolin was chosen as the target compound because, according to the pharmacopeia, it is a characteristic compound of tempuyung. The luteolin component is also utilized as a quality control marker in herbal medicines containing tempuyung components. The mobile phase with the L code was validated further, as it is a method development in fingerprint analysis for quality control.

3.2. Method Validation

3.2.1. Stability

The TLC separation method is an open system, and each step requires a particular delay time. This can affect the separation results due to the many external confounding factors that require stability testing. The first stability test parameter is the stability of the analyte during the chromatography process by developing two-dimensional chromatography. The TLC chromatogram

shows stable results because the stain is located on a diagonal line connecting the application position with the intersection of the two solvent fronts in both development directions (Figure 7).

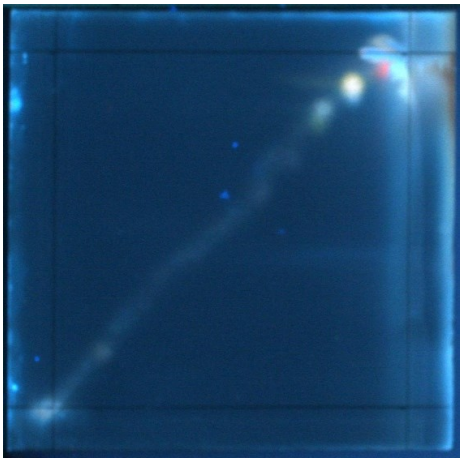

Figure 7. TLC chromatogram of analyte stability test after derivatization under UV 366 nm

The second stability parameter is the stability of the analyte resulting from visualization, which is determined by observing the visual chromatogram results at several time intervals. The experimental results were stable because there was no significant change in the band's color and the number of TLC stains (Figure 8).

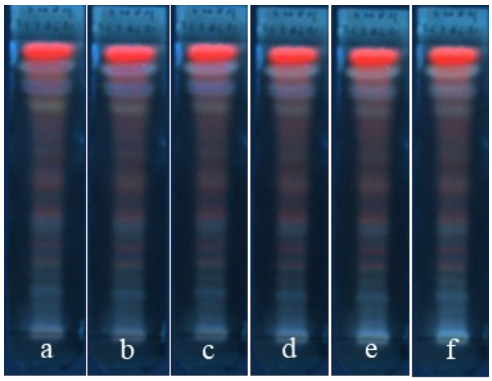

Figure 8. TLC chromatogram of tempuyung plant resulted from analyte stability test for a) 2 minutes, b) 5 minutes, c) 10 minutes, d) 20 minutes, e) 30 minutes, and f) 60 minutes

Figure 9. TLC chromatogram derivatized and detected with UV 366 nm from stability tests of (a)(b) analyte in the plates and (c)(d) analyte in solution

The third stability parameter is the stability of the analyte on the plate and sample solution. The test was conducted by comparing the (a) samples applied to the plate and left for 3 hours, (b) fresh samples, (c) samples left in the solution for 3 hours, and (d) fresh samples. The fingerprint pattern, color, quantity, and intensity of stains on the band did not change significantly, indicating that the separation remained stable (Figure 9).

3.2.2. Precision

The precision test was conducted using three plates tested on the same and different days. The results of the precision and intermediate precision tests showed fingerprint patterns, colors, numbers, and intensities that were not significantly different (Figure 10). The intraplate precision test yielded a mean R_f value for the luteolin (X) compound of 0.22 with a standard deviation of 0.0063. The mean R_f value for the luteolin compound obtained from the three-day precision testing results was 0.23, with a standard deviation of 0.0055. Based on these results, the precision and intermediate precision tests meet the acceptance requirements where the standard deviation R_f value for precision tests is less than 0.02 and for intermediate precision is less than 0.05 [16].

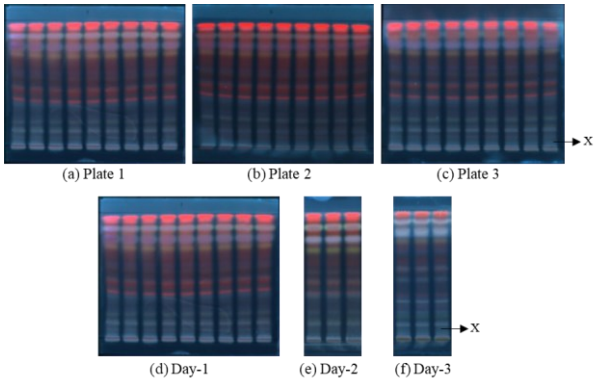

Figure 10. TLC profiles of precision measurements of (a) Plate 1, (b) Plate 2, (c) Plate 3, and the precision between (d) Day 1, (e) Day 2, (f) Day -3 after derivatization and detection with UV 366 nm

3.2.3. Specificity

Specificity was tested by comparing the fingerprint patterns of tempuyung and tapak liman plants because both have similar morphologies, such as shape, color, and texture. The test is conducted because the method is considered specific when testing samples with the same identity gives similar separation results, and vice versa [15]. TLC separation results showed differences in fingerprint patterns between tempuyung and tapak liman plants (Figure 11). The TLC stain band was visible in the tempuyung plant but not in the tapak liman at R_f = 0.78 (x). In contrast to tempuyung, tapak liman plants have strong red and bright blue stain bands at R_f 0.44 (z) and 0.64 (y), respectively. However, there are similarities in the bands of luteolin compounds, with an R_f value of 0.21 in both plants marked with a yellow line (Figure 11). These similarities make the development of this method not specific to the separation of luteolin compounds.

Figure 11. Fingerprint patterns of a) tempuyung, b) standard luteolin, and c) tapak liman after derivatization and detection with UV 366 nm

3.2.4. Robustness

The robustness test was conducted with two parameters: the robustness of the chamber type and the robustness of the development distance. Robustness tests on chamber types were performed using twin-trough and flat-bottom chambers. The results of the robustness test met the acceptance requirements. This can be seen from the resulting fingerprint patterns, which were not significantly different for the two types of chambers (Figures 12a & 12b), and the standard deviation R_f values obtained for both were less than 0.05. A robustness test on development distance was carried out by comparing the results of separation at 8 cm and 7 cm floating distances. The results of separating the two development distances met the acceptance requirements because the fingerprint patterns of the two are not significantly different (Figures 12a & 12b) with a standard deviation R_f of less than 0.05.

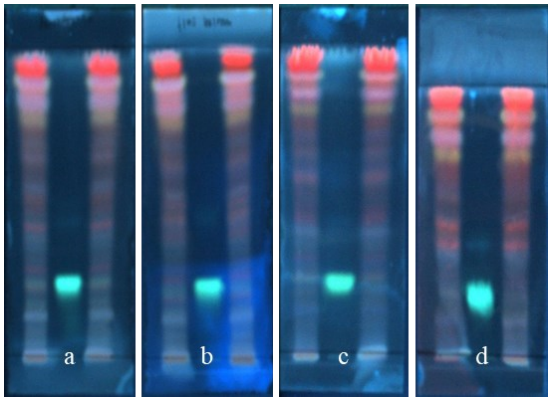

Figure 12. TLC profiles after derivatized and detected with UV 366 nm of development results using a) twin-through chambers, b) flat-bottom, c) 8 cm spacing, d) 7 cm spacing

3.3. TLC Separation Method Application

The validated TLC separation method was then used to separate tempuyung from three different locations, namely Malang (a1) (a2) (a3), Solo (b1) (b2) (b3), and Yogyakarta (c1) (c2) (c3). This determines whether this development method can be applied to tempuyung from different locations. The separated fingerprint profile (Figure 13) shows a pattern with the same bands and colors. The TLC fingerprint patterns from the three regions produced eleven bands with good and uniform separability. Luteolin-characterizing compounds are shown in band number 3 with an R_f value of 0.20. The difference is only found in the color intensity of the tempuyung fingerprint bands from Yogyakarta numbers 4 and 7, where the colors appear brighter than bands from other regions. The difference is thought to be due to differences in the concentration of a compound contained therein. Some factors causing these differences include different natural conditions, such as temperature, soil nutrients, postharvest treatment, and harvest period [19].

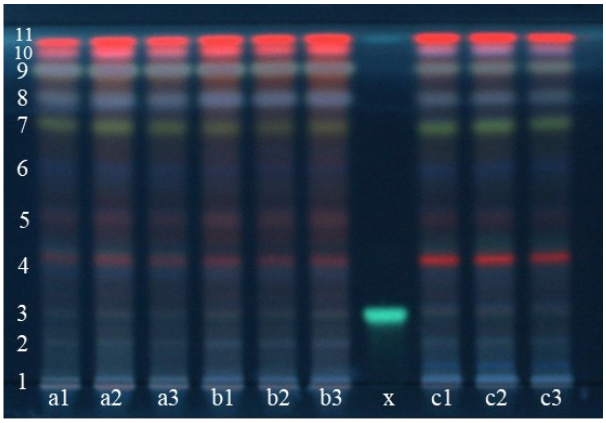

Figure 13. TLC profiles of tempuyung from three different locations: Malang (a1) (a2) (a3), Solo (b1) (b2) (b3), and Yogyakarta (c1) (c2) (c3)

The TLC fingerprint patterns of the three areas were then compared using a densitogram (Figure 14) to determine the level of component content in the samples. The resulting densitogram shows peak uniformity from three different locations. However, at peaks number 4 and 7 for the Yogyakarta area (d), the intensity was higher than for the other two regions (Malang (a) and Solo (b)). This shows that the compound content contained in sample (d) is much higher. This result corresponds to the band on the fingerprint pattern (Figure 13), where the band's color appears lighter.

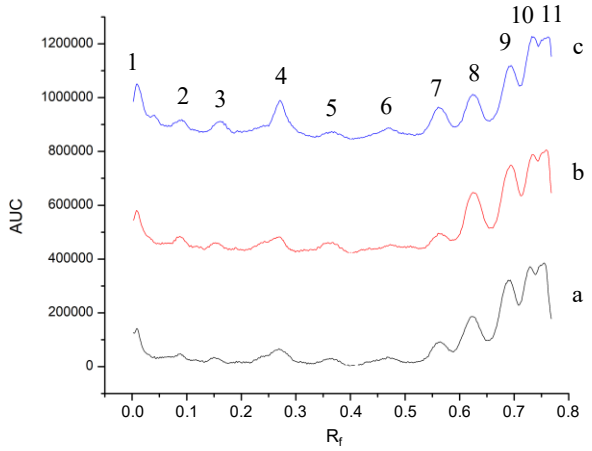

Figure 14. Densitogram of the tempuyung from a) Malang, b) Solo, c) Yogyakarta, based on the TLC fingerprint pattern in Figure 13

4. Conclusion

The TLC fingerprint analysis method development for tempuyung plants has been successfully implemented

and applied to tempuyung from Malang, Solo, and Yogyakarta. This method has been validated, and the experimental results for all test parameters have met the established criteria, allowing it to be utilized for tempuyung quality control. The optimum mobile phase chosen was a mixture of chloroform: ethyl acetate:dichloromethane: formic acid (7.5:2:0.5:0.1) because it could separate eleven bands with good separation results. These results were obtained using UV light at 366 nm and 10% sulfuric acid for derivatization.

References

[1] Xiu-Mei Li, Pei-Long Yang, Research progress of *Sonchus* species, *International Journal of Food Properties*, 21, 1, (2018), 147-157 https://doi.org/10.1080/10942912.2017.1415931

[2] Dwi Kusuma Wahyuni, Hery Purnobasuki, Eko Prasetyo Kuncoro, Wiwied Ekasari, Callus induction of *Sonchus arvensis* L. and its antiplasmodial activity, *African Journal of Infectious Diseases*, 14, 1, (2020), 1-7 https://doi.org/10.21010/ajid.v14i1.1

[3] Tatik Raisawati, Maya Melati, Sandra Arifin Aziz, Muhamad Rafi, Total Flavonoid Content and Antioxidant Activity in Leaves of Ten Cultivated *Sonchus Arvensis* L. Accessions, *Plant Archives*, 20, 1, (2020), 1785-1792

[4] Suryani, E. Y. Sukandar, M. Insanu, N. F. Kurniati, Evaluation Effect of Polarity of The Compound from *Sonchus arvensis* (Linn.) Leaves as Hypertension inhibitor, *Rasayan Journal of Chemistry*, 13, 3, (2020), 1807-1816

[5] Roya Karamian, Mostafa Asadbegy, Antioxidant activity, total phenolic and flavonoid contents of three Onobrychis species from Iran, *Pharmaceutical Sciences*, 22, 2, (2016), 112-119 https://doi.org/10.15171/PS.2016.18

[6] Min Rahminiwati, Raudhatul Jannah, Kusdiantoro Mohamad, Studi Perbandingan Efek Diuretik dan Antiinflamasi Gabungan Ekstrak Sidaguri, Tempuyung dan Seledri pada Tikus, *Fitofarmaka: Jurnal Ilmiah Farmasi*, 11, 2, (2021), 118-128 https://doi.org/10.33751/jf.v11i2.2999

[7] Tian-yang Wang, Qing Li, Kai-shun Bi, Bioactive flavonoids in medicinal plants: Structure, activity and biological fate, *Asian Journal of Pharmaceutical Sciences*, 13, 1, (2018), 12-23 https://doi.org/10.1016/j.ajps.2017.08.004

[8] Buyung Rukmantara Susena Putra, Dewi Kusrini, Enny Fachriyah, Isolasi Senyawa Antioksidan dari Fraksi Etil Asetat Daun Tempuyung (*Sonchus arvensis* L.), *Jurnal Kimia Sains dan Aplikasi*, 16, 3, (2013), 69-72 https://doi.org/10.14710/jksa.16.3.69-72

[9] Rifki Husnul Khuluk, Amalia Yunita, Eti Rohaeti, Utami Dyah Syafitri, Roza Linda, Lee Wah Lim, Toyohide Takeuchi, Mohamad Rafi, An HPLC-DAD method to quantify flavonoids in *sonchus arvensis* and able to classify the plant parts and their geographical area through principal component analysis, *Separations*, 8, 2, (2021), 12 https://doi.org/10.3390/separations8020012

[10] Departemen Kesehatan Republik Indonesia, in, Departemen Kesehatan Republik Indonesia, Jakarta, 2017, p. 186-187

[11] ZhengXiang Xia, JingYu Liang, Steroids and phenols from Sonchus arvensis, *Chinese Journal of Natural Medicines*, 8, 4, (2010), 267-269

[12] Tapan Seal, Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India, *Journal of Applied Pharmaceutical Science*, 6, 2, (2016), 157-166 http://dx.doi.org/10.7324/JAPS.2016.60225

[13] Yilin Chen, Linnan Li, Rui Xu, Fan Li, Lihua Gu, Huwei Liu, Zhengtao Wang, Li Yang, Characterization of natural herbal medicines by thin-layer chromatography combined with laser ablation-assisted direct analysis in real-time mass spectrometry, *Journal of Chromatography A*, 1654, (2021), 462461 https://doi.org/10.1016/j.chroma.2021.462461

[14] Nina Irmayanti Harahap, Skrining dan karakterisasi simplisia daun tempuyung (*Sonchus arvensis*. L), *Jurnal Ilmiah Farmasi Imelda*, 3, 2, (2019), 45-51 https://doi.org/10.52943/jifarmasi.v3i2.212

[15] Mohamad Rafi, Rudi Heryanto, Dewi Anggraini Septiningsih, in, IPB Press, 2017,

[16] Eike Reich, Anne Schibli, in: High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants, Georg Thieme Verlag KG, Stuttgart, 2007, https://www.doi.org/10.1055/b-0034-65188

[17] Ignacio Cabezudo, Mario O. Salazar, I. Ayelen Ramallo, Ricardo L. E. Furlan, Effect-directed analysis in food by thin-layer chromatography assays, *Food Chemistry*, 390, (2022), 132937 https://doi.org/10.1016/j.foodchem.2022.132937

[18] Makmum Syafi'i, Eti Rohaeti, Wulan Tri Wahyuni, Mohamad Rafi, Dewi Anggraini Septaningsih, Analisis Sidik Jari Kromatografi Lapis Tipis Rimpang Temu Mangga (*Curcuma mangga*), *Jurnal Jamu Indonesia*, 3, 3, (2018), 109-115 https://doi.org/10.29244/jji.v3i3.68

[19] Wulan Tri Wahyuni, Meri Saharah, Zulhan Arif, Mohamad Rafi, Thin layer chromatographic fingerprint and chemometrics analysis for identification of Phyllanthus niruri from its related species, *Journal of the Indonesian Chemical Society*, 3, 1, (2020), 47-52