# Determination of Quercetin in Extracts and Herbal Products of *Phyllanthus niruri* by TLC Densitometry Method

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## Abstract

*Phyllanthus niruri* has many pharmacological activities such as antioxidant, anti-inflammatory and anticancer. *Phyllanthus niruri* has been widely developed from extracts into herbal products. The flavonoid quercetin became an analytical marker compound to ensure the quality of *P. niruri* extracts in the Indonesian herbal pharmacopeia II. This study aimed to determine the levels of quercetin in extract and herbal products using the TLC Densitometry method. TLC Densitometry method using chloroform: ethyl acetate: formic acid (5:4:1) as the mobile phase and silica GF254 as the stationary phase. The Rf value of the quercetin compound obtained is 0.50. The Rs quercetin is 1.56 in the extract and 2.55 in the product. The maximum wavelength for quercetin is 366 nm. The results of quercetin levels were 3.579% in the extract and 0.0412% in herbal products. The TLC densitometry method can analyze quercetin in extracts and herbal products.

***Keywords**: Quercetin, Phyllanthus niruri L., TLC-densitometry, herbal products*

## Background

*Phyllanthus niruri* has become a traditional medicine in several country for example in Indonesia, India and Malaysia (Seyed, 2019). *Phyllanthus niruri* has many pharmacological activities such as antioxidant (Colpo *et al*., 2014), antiplasmodial (Ifeoma *et al*., 2013), repairing wound (Shanbhag *et al*., 2010), antimicrobial (Pathania *et al*., 2022), and anti-inflammatory and antinociceptive (Porto *et al*., 2013). There were flavonoid compounds in *Phyllanthus niruri* such as rutin, quercetin, quercitrin, kaempferol and astragalin which has pharmacological activity (Bagalkotkar *et al*., 2010).

Some people in developing countries uses medicinal plant for resolving their health problem because efficacy, low risk toxicity and minimal side effect. *Phyllanthus niruri* which has many pharmacological activities was widely developed from extracts into herbal products (Porto *et al*., 2013; Pathania *et al*., 2022). Quercetin, one of the active compounds in *Phyllanthus niruri*, has pharmacologycal activites such as antihyperuricemia and gouty arthritis (Nutmakul, 2022), antioxidant, antiviral, antihypertension, antitumor (Alizadeh & Ebrahimzadeh, 2022). Extraction have aim to collect active metabolite from raw materials. Some extraction method developed to obtain efficiency extraction (Zhang *et al*., 2018; Ihsan *et al*., 2018).

Quercetin can be used as analytical marker compound to ensure quality *Phyllanthus niruri* extract. *Phyllanthus niruri* herb extract must contains a total flavonoid not less than 3.20% as quercetin (Indonesian Herbal Pharmacopoeia, 2017). Analytical marker compound was compound in plant that dominant in plant, easy analysis or have important activities (Ihsan *et al*., 2020). Some previous analytical method to analysis quercetin were spectrophotometry UV-Vis (Soylak *et al*., 2020), TLC (Ihsan *et al*., 2019), HPLC (Dinakaran *et al*., 2018), LC-MS (Kumar *et al*., 2015), HPTLC (Amir *et al*., 2013). TLC densitometry method was a simple, selective and inexpensive method for routine analysis in quality assurance of traditional medicines (Hafid *et al*., 2015). The purpose of this study was to determine the levels of quercetin in extract and herbal product using the TLC Densitometry method.

## Materials and Methods

### *Materials*

Quercetin (Sigma Aldrich), chloroform (Merck), methanol (Merck), acetone (Merck), formic acid (Merck), ethyl acetate (Merck), and aquadest. *Phyllanthus niruri* simplicia from Balai Materia Medica (Malang, Indonesia).

### *Instrument*

TLC scanner (CAMAG), chamber (CAMAG), plate TLC silica GF254, rotary evaporator (IKA), stirrer (IKA), UV lamp (CAMAG), analytical balance (Shimadzu AUW 220).

### *Extraction method*

300 g of *Phyllanthus niruri* L. was dissolved in 1000 ml ethanol 96%. Then stirred 30 minutes. This solution was macerated for 24 hours (This Procedure was repeated 3 times). The collected filtrate was evaporated with a rotary evaporator until it became an extract.

### *Preparation of standard solution*

Stock solution was prepared 200 ppm. Then diluted to five concentration.

### *Preparation of test solution*

100 mg extract was dissolved with ethanol 96% in 25 ml volumetric flask. This solution was filtered before 3 µl applied in TLC plate. 1000 mg was dissolved with ethanol 96% in 10 ml volumetric flask. This solution was filtered before 8 µl applied in TLC plate.

### *Optimization of mobile phase*

Three composition of mobile phase was evaluated for selection the best separation of quercetin in sample. The composition of mobile phase was shown in **Table 1**.

**Table 1. The composition mobile phase optimization**

| The compostion of mobile phase | Rf of quercetin standard | Quercetin resolution in extract | Quercetin resolution in herbal product |
|---|---|---|---|
| Composition I (Chloroform : Methanol : Water = 80:12:2) | 0.23 | 1.06 | 1.37 |
| Composition II (Chloroform : Acetone : Methanol : formic acid = 28 : 9 : 2: 1) | 0.42 | 1.33 | 1.17 |
| Composition III (Chloroform : ethyl acetate : formic acid = 5 : 4 : 1) | 0.50 | 1.56 | 2.55 |

***Qualitative analysis TLC method***
Retardation factor (Rf) and Resolution (Rs) were used to evaluated separation. Spot quercetin standard, extract sample and herbal product were evaluated by scanning at 200-400 nm.

***Quantitative analysis TLC method***
The curve calibration was used to calculate concentration quercetin in sample. Five concentration of quercetin standard was spotted 2 µl and was scanned at 366 nm.

## Result and Discussion

The yield of the *Phyllanthus niruri* extraction method in this study was 36%. Extraction used ethanol 96% because quercetin quite soluble in alcohol. Extraction of secondary metabolites was influenced by the type of solvent, the solvent to solid ratio, the extraction duration, and the amount of maceration.
Mobile phase optimization aimed to obtain the best mobile phase composition that can separate quercetin from another component in sample. The composition and results of Retardation factor (Rf) and Resolution (Rs) were shown in **Table 1**. The Composition III (Chloroform: ethyl acetate: formic acid = 5:4 :1) was selected because show the best quercetin resolution in extract and herbal product. The Rf value of standard quercetin was 0.5. This Rf also meet the requirement between 0.2-0.8. The separation of quercetin in extract and herbal product of *Phyllanthus niruri* by composition III of mobile phase was shown in **Figure 1**.

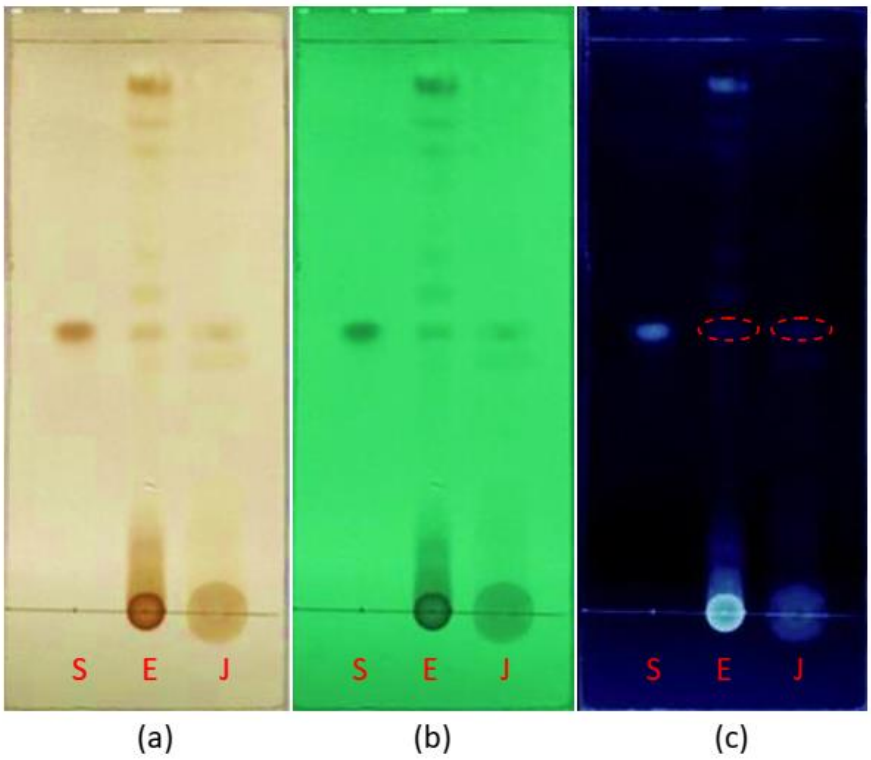


**Figure 1. Separation quercetin standard (S), extract (E), and herbal product (J), (a) detected by visual, (b) detected at 254 nm, (c) detected at 366 nm**

This selectivity in this method showed that analyte quercetin can separate from another component in the sample. Chromatogram and spectrum quercetin from extract and product herbal was shown at **Figure 2** and **3**. The selectivity quercetin in extract and herbal product was shown by comparing spectrum shape and maximum wavelength of quercetin. The maximum wavelength of quercetin was 366 nm.

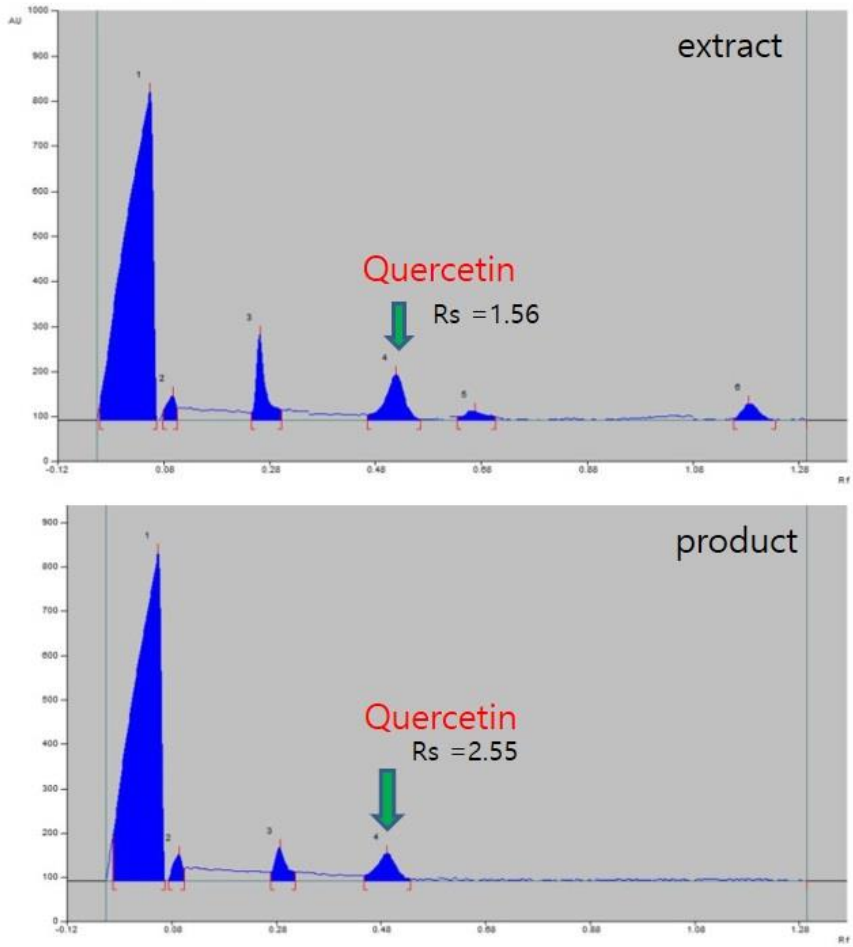


**Figure 2. Chromatogram of extract and herbal product**

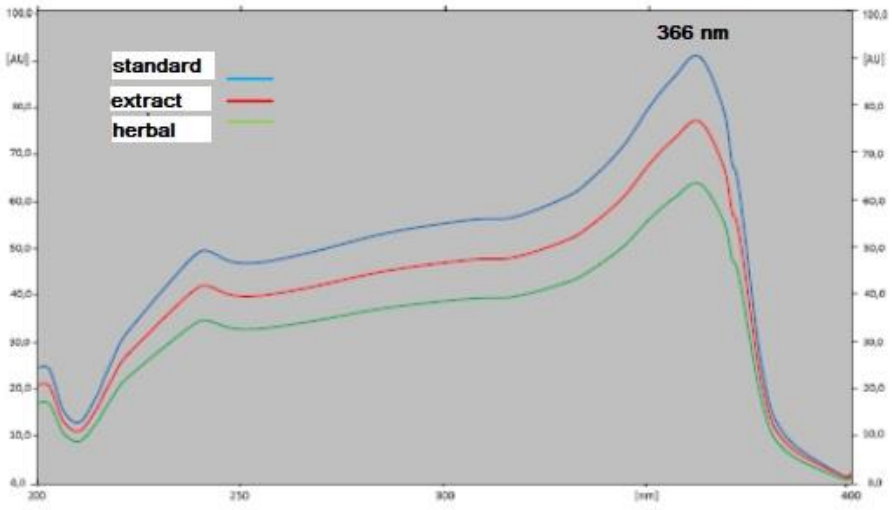


**Figure 3. Comparison of spectrum from quercetin standard, quercetin in extract and herbal product**

Quantitative analysis of quercetin in the sample was carried out by calibration curve of five standard concentration of quercetin. Regression equation was y = 14576x - 2918. The coefficient correlation was 0.9998. The results of concentration quercetin in extract and herbal product was shown at **Table 2**.

**Table 2. The concentration quercetin in extract and herbal product**

| Sample | Area | Quercetin in 25 ml (mg) | % W/W | %W/W (X ± SD) |
|---|---|---|---|---|
| Extract | 3,346.40 | 3.581 | 3.581 | 3.5 ± 0.05 |
| | 3,434.10 | 3.632 | 3.632 | |
| | 3,251.80 | 3.527 | 3.527 | |
| **Sample** | **Area** | **Quercetin in 10 ml (mg)** | **% W/W** | **%W/W (X ± SD)** |
| Herbal product | 1,871.31 | 0.410 | 0.0410 | 0.041 ± 0.0002 |
| | 1,892.20 | 0.413 | 0.0413 | |
| | 1,910.10 | 0.414 | 0.0414 | |

The result of the determination of the quercetin content in extract was 3.5%. The concentration quercetin in the extract meet the Indonesian herbal pharmacopoeia II. *Phyllanthus niruri* L. herb extract contains a total flavonoid not less than 3.20% calculated as quercetin. This extraction method can be applied to production extract from *Phyllanthus niruri*. The result of the determination of the quercetin content in extract was 0.041%. Each herbal product had certain specifications. This is based on consideration of herbal product indication.

## Conclusion

The thin layer chromatography densitometry method can be used for analysis of quercetin in extract and herbal product of *Phyllanthus niruri*.

## Acknowledgment

We are very grateful to Balai Materia Medica Malang, East Java for their support in this research.

## References

Alizadeh, S. R., & Ebrahimzadeh, M. A. (2022). Quercetin derivatives: Drug design, development, and biological activities, a review. *European Journal of Medicinal Chemistry*, **229**, 114068. https://doi.org/10.1016/j.ejmech.2021.114068

Amir, M. Mujeeb, M., Ahmad, S., Akhtar, M., & Ashraf, K. (2013). Design expert-supported development and validation of HPTLC method: An application in simultaneous estimation of quercetin and rutin in Punica granatum, Tamarindus indica and Prunus domestica. *Pharmaceutical Methods*, **4**(2), 62–67. https://doi.org/10.1016/j.phme.2013.12.004

Bagalkotkar, G., Sagineedu, S. R., Saad, M. S., & Stanslas, J. (2010). Phytochemicals from Phyllanthus niruri Linn. and their pharmacological properties: a review. *Journal of Pharmacy and Pharmacology*, **58**(12), 1559–1570. https://doi.org/10.1211/jpp.58.12.0001

Colpo, E., Vilanova, C. D. D. A., Pereira, R. P., Reetz, L. G. B., Oliveira, L., Farias, I. L. G., et al. (2014). Antioxidant effects of Phyllanthus niruri tea on healthy subjects. *Asian Pacific Journal of Tropical Medicine*, **7**(2), 113–118. https://doi.org/10.1016/S1995-7645(14)60005-5

Dinakaran, S. K., Sujiya, B., & Avasarala, H. (2018). Profiling and determination of phenolic compounds in Indian marketed hepatoprotective polyherbal formulations and their comparative evaluation. *Journal of Ayurveda and Integrative Medicine*, **9**(1), 3–12. https://doi.org/10.1016/j.jaim.2016.12.006

Hafid, A. F., Rifai, B., Tumewu, L., Widiastuti, E., Dachliyati, L., Primaharinastiti, R., *et al*. (2015). Andrographolide determination of Andrographis paniculata extracts, ethyl acetate

fractions and tablets by thin layer chromatography. *Journal of Chemical and Pharmaceutical Research*, **7**(12), 557-561.

Ifeoma, O., Samuel, O., Itohan, A. M., & Adeola, S. O. (2013). Isolation, fractionation and evaluation of the antiplasmodial properties of Phyllanthus niruri resident in its chloroform fraction. *Asian Pacific Journal of Tropical Medicine*, **6**(3), 169–175. https://doi.org/10.1016/S1995-7645(13)60018-8

Ihsan, B. R. P., Hafid, A. F., Primaharinastiti, R., Widyawaruyanti, A., & Yuwono, M. (2020). Development and validation of hplc method for determination of andrographolide in raw material and tablet of ethyl acetate fractions of Andrographis paniculata. *Research Journal of Pharmacy and Technology*, **13**(9), 4291. https://doi.org/10.5958/0974-360X.2020.00758.1

Ihsan, B. R. P., Maysaroh, I., & Nurhayati, I. P. (2018). Validasi Metode Ultra High Performance Chromatography Double Mass Spectrometry (UHPLC-MS/MS) untuk Analisis Kurkumin pada Ekstrak Etanol Kunyit (Curcuma longa) dengan Berbagai Perbandingan. *Pharmaceutical Journal of Indonesia*, **4**(1), 29-34.

Ihsan, B. R. P., Rahmani, P. A., & Shalas, A. F. (2019). Validasi Metode KLT-Densitometri untuk Analisis Kuersetin dalam Ekstrak dan Produk Jamu yang Mengandung Daun Jambu Biji (Psidium guajava L.). *Pharmaceutical Journal of Indonesia*, **5**(1), 45-51.

Kumar, S., Chandra, P., Bajpai, V., Singh, A., Srivastava, M., Mishra, D. K., *et al*. (2015). Rapid qualitative and quantitative analysis of bioactive compounds from Phyllanthus amarus using LC/MS/MS techniques. *Industrial Crops and Products*, **69**, 143–152. https://doi.org/10.1016/j.indcrop.2015.02.012

Nutmakul, T. (2022). A review on benefits of quercetin in hyperuricemia and gouty arthritis. *Saudi Pharmaceutical Journal*, **30**(7), 918–926. https://doi.org/10.1016/j.jsps.2022.04.013

Pathania, R., Najda, A., Chawla, P., Kaushik, R., & Khan, M. A. (2022). Low-energy assisted sodium alginate stabilized Phyllanthus niruri extract nanoemulsion: Characterization, in vitro antioxidant and antimicrobial application. *Biotechnology Reports*, **33**, e00711. https://doi.org/10.1016/j.btre.2022.e00711

Porto, C. R. C., Soares, L. A. L., Souza, T. P., Petrovick, P. R., Lyra, I. L., Junior, R. F. A., *et al*. (2013). Anti-inflammatory and antinociceptive activities of Phyllanthus niruri spray-dried standardized extract. *Revista Brasileira de Farmacognosia*, **23**(1), 138–144. Available at: https://doi.org/10.1590/S0102-695X2013005000004

Seyed, M. A. (2019). A comprehensive review on Phyllanthus derived natural products as potential chemotherapeutic and immunomodulators for a wide range of human diseases. *Biocatalysis and Agricultural Biotechnology*, **17**, 529–537. https://doi.org/10.1016/j.bcab.2019.01.008

Shanbhag, T., Amuthan, A., Shenoy, S., & Sudhakar. (2010). Effect of Phyllanthus niruri. Linn on burn wound in rats. *Asian Pacific Journal of Tropical Medicine*, **3**(2), 105–108. https://doi.org/10.1016/S1995-7645(10)60045-4

Soylak, M., Ozdemir, B., & Yilmaz, E. (2020). An environmentally friendly and novel amine-based liquid phase microextraction of quercetin in food samples prior to its determination by UV–vis spectrophotometry. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **243**, 118806. https://doi.org/10.1016/j.saa.2020.118806

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, **13**, 20. https://doi.org/10.1186/s13020-018-0177-x