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Establishment of quality standards for *Morinda citrifolia* L. Rubiaceae harvested at Mephydica Co., LTD.

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ABSTRACT

Background: Noni fruit (Morinda citrifolia) is widely used in traditional medicine for health support; however, quantitative scientific data on its active constituents remain limited. Previous studies have shown that flavonoids, polyphenols, coumarins, and scopoletin contribute to the biological activities of Noni fruit, but their contents are strongly influenced by raw material origin, ripeness, and processing methods. The absence of quality and dosage standardization may affect therapeutic effectiveness and safety. Objective: This study aimed to perform preliminary phytochemical screening, thin-layer chromatography (TLC), and quantitative determination of total flavonoids, total polyphenols, and scopoletin in Noni fruit. Materials and methods: Noni fruits were collected from Mephydica Co., Ltd. The chemical composition was evaluated using preliminary phytochemical screening and TLC. Total flavonoid content (TFC) and total polyphenol content (TPC) were determined using the aluminum chloride and Folin-Ciocalteu methods, respectively. Scopoletin was quantified by UV-Vis spectrophotometry. Results: Phytochemical screening revealed the presence of diverse bioactive compounds, including coumarins, saponins, and reducing agents. TLC analysis showed a chromatographic spot corresponding to the scopoletin reference standard. The TFC of the methanolic extract was 25.232 ± 0.023 mg QE/g dry extract, while the TPC was 6.413 ± 0.112 mg GAE/g dry extract. The scopoletin content was determined as 1.636 ± 0.05 mg/g dry extract. Conclusion: The study provides quantitative data on flavonoids, polyphenols, and scopoletin in methanolic extracts of Noni fruit, supporting its traditional use and contributing to quality standardization and future applied research.

***Keywords**: Morinda citrifolia, total flavonoid content, total polyphenol, scopoletin, UV-Vis*

1. INTRODUCTION

In recent years, Noni fruit (*Morinda citrifolia*) has been widely consumed in various forms, including fresh fruit, juice, and traditionally processed products. This trend is largely driven by the widespread belief in its potential health benefits, such as supporting the management of certain conditions, including hemorrhage, obesity, and osteoarthritis [1, 2]. However, despite its increasing popularity in community use, the consumption of Noni is not yet fully supported by sufficient quantitative scientific data regarding its active constituents, particularly in raw materials used directly by consumers. Previous studies have reported that *M. citrifolia* contains numerous bioactive compounds, among which flavonoids, polyphenols, and scopoletin are considered to be associated with antioxidant, anti-inflammatory, and physiological regulatory activities [3 - 6]. Never-theless, the concentrations of these compounds may vary considerably depending on factors such as geographical origin, fruit maturity, and processing or extraction methods, resulting in inconsistencies among reported results. In addition, many previous studies have focused on individual groups of compounds or have employed different analytical techniques, making a comprehensive comparison of the chemical composition of Noni fruit difficult. Therefore, the simultaneous determination of total flavonoids, total poly-phenols, and scopoletin in *M. citrifolia* using ap-propriate analytical methods is necessary to provide additional scientific data for evaluating its chemical composition and to support the rational utilization of this medicinal plant resource.

2. MATERIALS AND METHODS

2.1 Research materials

Ripe fruits of *Morinda citrifolia* L. were collected in August 2025 from Mephydica Co., Ltd. The botanical

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raw materials were harvested at full ripeness to ensure optimal phytochemical content. After collection, the samples were transported and stored at the Faculty of Pharmacy, Hong Bang International University, under appropriate conditions prior to analysis.

2.1.1. Chemicals, equipment

The instruments used in this study included a Shimadzu UV-1800 UV-Vis spectrophotometer (Japan); Elmasonic S Ultrasonic tank (Germany); Micropipet 10 - 100 µL, Micropipet 100 - 1000 µL (Germany); Scout Pro Technical Scales (Germany), and TLC Silica Gel 60 F254 (Germany).

All chemicals and reagents used were of analytical grade. The chemicals employed in this study are presented in Table 1.

Table 1. Chemicals used for the study

Chemicals	Origin
Quercetin ≥ 95% (HPLC)	Germany
Gallic acid	Germany
Scopoletin ≥ 97.72 (HPLC)	Germany
Folin-Ciocalteu	Germany
Methanol	China
Chloroform	China
Ethyl Acetate	China
Formic Acid	China
$AlCl_3$	VietNam
Distilled water	VietNam

2.2. Methods

2.2.1. Extraction

Fresh Noni fruit (moisture content: 88%) was collected and dried at 70°C. The dried material was then pulverized into powder and used as the experimental material. The powder was placed into a beaker, and methanol was added as the solvent at a solid-to-liquid ratio of 1:10 (w/v). The mixture was sealed and subjected to ultrasonic-assisted extraction for 40 minutes. Following the first extraction, the residue underwent two additional extraction cycles under the same conditions to maximize the recovery of active ingredients. The completeness of the extraction process was monitored by thin-layer chro-matography (TLC). The methanol extract (moisture content: 10.24%) is used for thin-layer chro-matography and quantitative processes. A similar extraction process was employed using water and ethanol as solvents for phytochemical investigation and qualitative analysis.

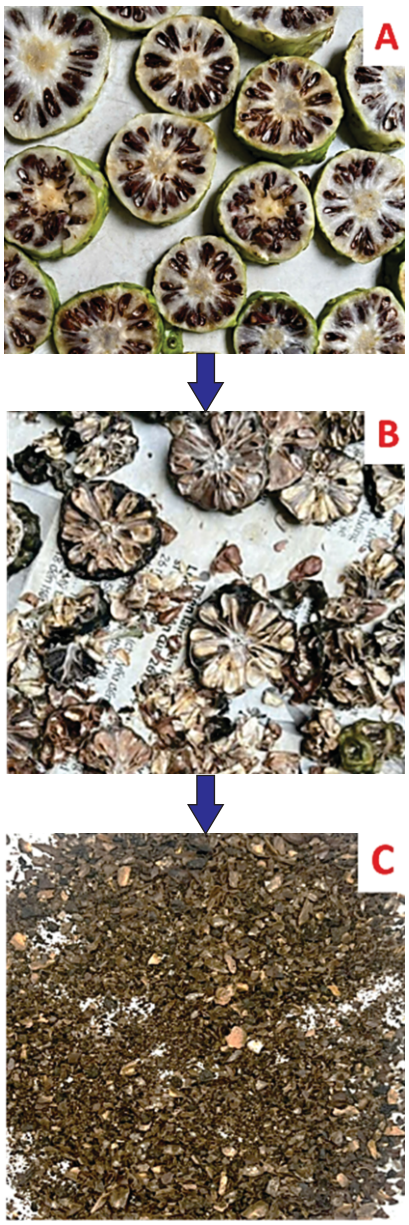

Figure 1. Noni fruit processing
A: Fresh Noni fruit; B: Dried Noni fruit; C: Dried Noni fruit powder

2.2.2. Qualitative analysis

Phytochemical investigation

The chemical composition of the Noni fruit extract was comprehensively analyzed to identify its bioactive constituents following I. Ciulei's method [7].

Ethanolic and aqueous extracts were prepared to conduct characteristic chemical reactions aimed at identifying potential bioactive compounds within the Noni fruit matrix. Typical procedures included specific qualitative tests for flavonoids, alkaloids, coumarins, and saponins,...

Qualitative determination using Thin Layer Chromatography (TLC)

A methanol extract was utilized for TLC analysis, using

scopoletin as an external standard for comparative identification. The mobile phase consisted of a mixture of chloroform and ethyl acetate in a volume ratio of 9:1 (v/v), with the addition of 1 drop of formic acid to optimize separation. The resulting chromatograms were recorded and evaluated through a direct comparison of the migration patterns and R_f values of the test extracts against those of the authentic scopoletin standard.

The chromatographic bands were visualized under UV light at wavelengths of 254 and 365 nm. Subsequently, the plate was derivatized by dipping into vanillin-sulfuric (VS) acid reagent, followed by a secondary observation under 365 nm UV light to characterize the colored fluorescence of the constituents.

Calculation formula:

$$R_f = \frac{A}{B}$$

In which: R_f: Relative mobility.
A: Distance traveled by the substance (cm).
B: Distance traveled by the solvent (cm).

2.2.3. Determination of total flavonoid content

The UV-Vis absorption spectrum was scanned in the wavelength range of 400 - 600 nm to determine the maximum absorption wavelength (λmax) of the flavonoid-aluminum chloride ($AlCl_3$) complex. Based on the selected wavelength, a linear calibration curve was constructed using quercetin as the reference standard by measuring the absorbance of standard solutions at different concentrations [8].

Blank sample: Exactly 2 mL of methanol, 1 mL of distilled water, and 1 mL of 5% $AlCl_3$ solution were mixed in a test tube. The mixture was shaken thoroughly and transferred into a quartz cuvette for absorbance measurement at the selected wavelength.

Standard sample: Approximately 25 mg of quercetin standard was accurately weighed and dissolved in methanol to obtain a stock solution with a concentration of 0.5 mg/mL. The stock solution was serially diluted with methanol to prepare a series of working standard solutions at appropriate concentrations.

An aliquot of 2 mL of each standard solution was transferred into a test tube, followed by the addition of 1 mL of distilled water and 1 mL of 5% $AlCl_3$ solution. After thorough mixing, the solution was transferred into a quartz cuvette, and its absorbance was measured at the predetermined wavelength. The calibration curve was established by plotting absorbance versus quercetin concentration.

Sample: From the extracted solution, diluted with methanol for quantification. A 2 mL aliquot of the sample solution was mixed with 1 mL of distilled water and 1 mL of 5% $AlCl_3$ solution. After mixing, the solution was transferred into a quartz cuvette and measured at the selected wavelength.

The total flavonoid content of the samples was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of sample (mg QE/g). Each sample was repeated 3 times. All concentrations (C) obtained from the calibration curves were expressed in µg/mL. Therefore, a conversion factor of 1,000 was applied to express the final results in mg per gram of dry extract.

$$TFC = \frac{C \times k \times V}{1000 \times m \times (1-MC)}$$

Where: TFC: Total flavonoid content (mg QE/g dry extract).
C: Concentration obtained from the calibration curve (µg/mL).
k: Dilution factor.
V: Total volume of extract (mL).
m: Sample weight (g).
MC: Moisture content (%).

2.2.4. Determination of total polyphenol content

The UV-Vis absorption spectrum was scanned in the wavelength range of 600 - 800 nm to determine the maximum absorption wavelength (λmax) of the complex formed between phenolic compounds and the Folin-Ciocalteu (F-C) reagent [9]. Based on the selected wavelength, a linear calibration curve was constructed using gallic acid as the reference standard.

Sample: The methanol extract was appropriately diluted with methanol. Exactly 2 mL of the resulting solution was transferred into a 25 mL volumetric flask. Distilled water and 10% Folin-Ciocalteu reagent were added, followed by the addition of 29% Na_2CO_3 solution to the volume according to the reaction procedure. The mixture was shaken thoroughly and allowed to react for an appropriate time before measuring absorbance at the selected wavelength. Each sample was repeated 3 times.

Blank sample: The blank consisted of methanol, distilled water, Folin-Ciocalteu reagent, and 29% Na_2CO_3 solution in the ratio of 2:10:1:12 (mL), respectively. The blank was prepared in the same manner as the test sample but without the extract.

Preparation of standard sample**:** A series of gallic acid standard solutions with concentrations ranging from 0.1 to 0.5 mg/mL was prepared by appropriate dilution of the stock solution with methanol.

Procedure: Each standard solution was treated similarly to the test sample with Folin-Ciocalteu reagent and Na_2CO_3 solution. After incubation, the absorbance was measured at the predetermined maximum wavelength. The calibration curve was established by plotting absorbance against gallic acid concentration. All concentrations (C) obtained from the calibration curves were expressed in µg/mL. Therefore, a conversion factor of 1000 was applied to express the final results in mg per gram of dry extract.

$$TPC = \frac{C \times k \times V}{1000 \times m \times (1\text{-}MC)}$$

Where:
TPC: Total Polyphenol Content (mg GAE/g dry extract).
C: Concentration from the gallic acid calibration curve (µg/mL).
k: Dilution factor.
V: Total volume of extract (mL).
m: Sample weight (g).
MC: moisture content (%).

2.2.5. Quantification of scopoletin by UV-Vis spectroscopy

Sample preparation process

+ Test sample: The methanol extract was diluted with methanol to achieve the target concentration [10, 11].

The UV-Vis absorption spectrum was scanned in the wavelength range of 200 - 500 nm to determine the maximum absorption wavelength (λmax) of scopoletin [10, 11]. Based on the selected wavelength, a linear calibration curve was constructed using scopoletin as the reference standard.

Preparation of standard solutions: A stock standard solution of scopoletin was prepared in methanol. Serial dilutions were subsequently carried out to obtain a series of working standard solutions with decreasing concentrations at appropriate ratios. The absorbance of each standard solution was then measured at the selected maximum wavelength. The calibration curve was established by plotting absorbance versus scopoletin concentration.

Sample: The initial extract was appropriately diluted with methanol to obtain the required analytical concentration. The resulting solution was used for absorbance measurement at the selected wavelength. Each sample was repeated 3 times.

Blank sample: Methanol was used as the blank and measured under the same experimental conditions as the test samples. All concentrations (C) obtained from the calibration curves were expressed in µg/mL. Therefore, a conversion factor of 1000 was applied to express the final results in mg per gram of dry weight.

$$T_{Scopoletin} = \frac{C \times k \times V}{1000 \times m \times (1\text{-}MC)}$$

In which:
$T_{Scopoletin}$: Total scopoletin content (mg/g dry extract).
C: Sample concentration (µg/mL).
k: Dilution ratio.
V: Volume of the solution (mL).
m: Sample weight (g).
MC: Moisture content (%).

2.2.6. Statistical Analysis

The experimental data were calculated and statistically processed using Microsoft Excel software (Microsoft Corp., USA).

3. RESULTS

3.1 Results of qualitative

3.1.1. Results of phytochemical screening

The preliminary phytochemical screening results for both the aqueous and 96% ethanolic extracts are summarized in Table 2.

Findings reveal a diverse phytochemical profile in Mephydica's Noni fruit, exhibiting substantial potential for practical applications. Key constituents identified include coumarins, saponins, and reducing substances, among others.

Table 2. Phytochemical screening of aqueous and 96% ethanol extracts of Noni fruit

Phytochemical groups	Aqueous	96% Ethanol
Alkaloids	-	-
Coumarins	-	+++
Anthraquinone glycosides	-	-
Flavonoids	-	+
Cardiac glycosides	-	-
Anthocyanosids	-	-
Proanthocyanin	-	-
Tannin	+	+
Saponin	-	+++
Organic acids	-	+
Reducing agents	++	++
Polyuronic	++	-

In which: + present;++ moderately present; +++ highly abundant; - not detected

3.1.2. Thin-layer chromatography (TLC) results

Thin-layer chromatography results showed that the

methanol extract from Morinda citrifolia fruit (T) exhibited a spot with an average Rf value of approximately 0.35, nearly equivalent to the Rf value of the scopoletin standard (C) of 0.338 under the same chromatographic conditions. The similarity in R_f values between the test sample and the standard suggests the possible presence of scopoletin in the investigated Morinda citrifolia sample.

Under UV 254 nm, both the test sample (T) and the standard (C) showed a clear absorption spot at the corresponding position. Under UV 365 nm, the spot at this position fluoresced a characteristic blue color, consistent with the fluorescence characteristics of coumarin compounds. After treatment with the reagent and observation under UV 365 nm, this spot remained clearly visible, and its position matched that of the test sample and the standard.

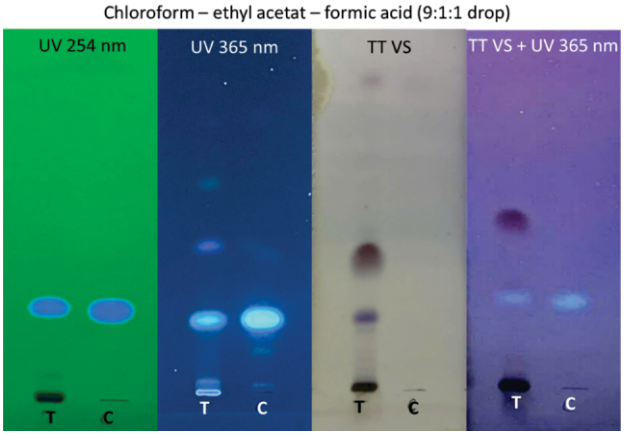

Figure 2. TLC of Noni Fruit

3.2. Results of quantification of total flavonoids by UV-Vis spectroscopy

The maximum absorption wavelengths (λ_{max}) for quercetin standards were identified at 430 nm following a spectral scan. These wavelengths were subsequently used to measure the absorbance of the remaining solutions within the established dilution series.

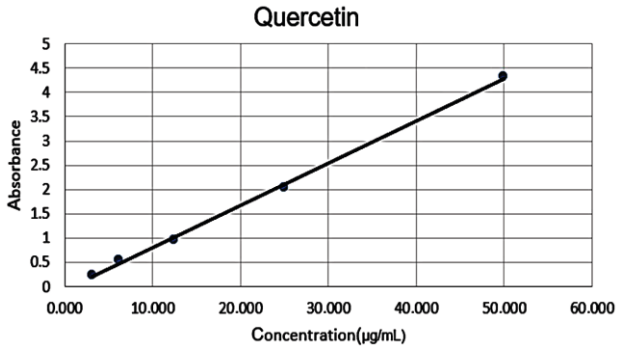

Figure 3. Standard curve of quercetin

The linear regression equation of the quercetin standard is: y = 0.087x - 0.0678 with a correlation coefficient R^2 = 0.9986. From the linear line according to each standard, the total flavonoid content of Noni methanol extract yielded about 25.232 ± 0.023 mg of GAE/g of dry extract.

3.3. Results of quantification of total polyphenols by UV-Vis spectroscopy

The maximum absorption wavelengths (λ_{max}) for gallic acid standards were identified at 760 nm, respectively, following a spectral scan.

These wavelengths were subsequently used to measure the absorbance of the remaining solutions within the established dilution series.

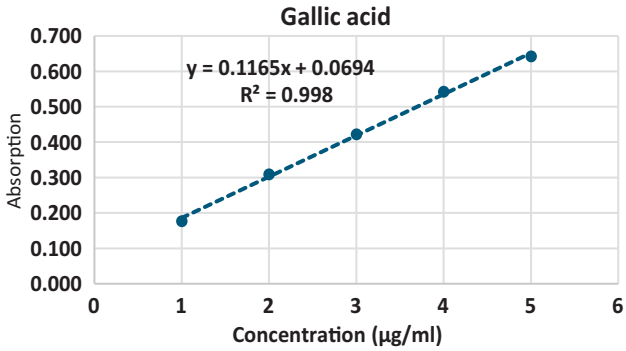

Figure 4. Standard curve of gallic acid

The linear regression equation of the gallic acid standard is: y = 0.1165x + 0.0694 with a correlation coefficient R^2 = 0.998. From the linear line according to each standard, the total polyphenol content of Noni methanol extract yielded about 6.413 ± 0.112 mg of GAE/g of dry extract.

3.4. Quantitative results of scopoletin in Noni fruit extract

After measuring samples in the dilution concentration range of the scopoletin standard, the obtained max wavelength is 344 nm, and the reference line is shown as follows.

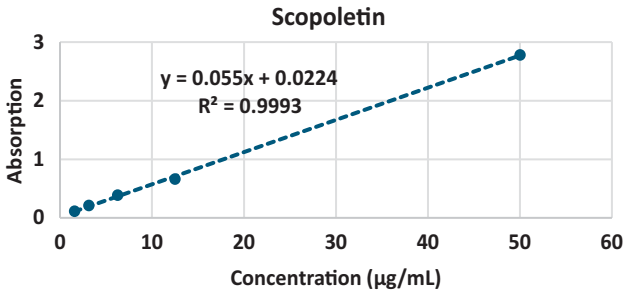

Figure 5. Standard curve of scopoletin

The linear regression equation of the scopoletin standard is: y = 0.055x + 0.0224 with a correlation coefficient R^2 = 0.9993. The scopoletin level of 1.636 ± 0.05 mg/g of dry extract reflects the pronounced presence of the coumarin group in the study sample.

4. DISCUSSION

The data on total polyphenol (TPC), total flavonoid (TFC), and scopoletin content in this study were determined from separate samples, analyzed using different methods and reference systems. Therefore, the content values are considered independent data, reflecting different groups of compounds in Noni, and are not intended for a direct quantitative comparison between the total content indicators. Compared to previous studies that focused on individual groups of compounds or used different analytical techniques, this study approached the simultaneous quantification of total polyphenols, total flavonoids, and scopoletin from Noni methanol extract using the UV-Vis method. This approach is feasible and highly applicable in the assessment and quality control of medicinal herbs.

With a content of 25.232 ± 0.023 mg QE/g of dry extract, this study shows that flavonoids are one of the most pronounced and important components present in Noni fruits. This result is consistent with previous analyses, such as the study by Xiaoai Chen et al. (2024) (approximately 15.32 mg/g), despite the fact that flavonoid accumulation can vary with growth stages and environmental conditions. The use of methanol is a key factor in optimizing the flavonoid extraction process. Due to its similar polarity to flavonoid glycosides, methanol facilitates the recovery of this group of active ingredients from Noni fruit in the most efficient and comprehensive manner [12].

The total polyphenol content (TPC) obtained in this study was 6.413 ± 0.112 mg GAE/g of dry extract when extracted with methanol, suggesting that phenolic compounds are not the dominant group in Noni fruit. This accurately reflects the chemical characteristics of *M. citrifolia*, as numerous studies on Noni fruit samples also report moderate TPC when employing UV-Vis spectrophotometry quantification techniques, such as the study by Qianxin Li et al. (2025) (approximately 10.68 - 10.81 mg/g of dry weight when extracted with 80% methanol and water, respectively) [13].

With a scopoletin content of 1.636 ± 0.05 mg/g of dry extract, this value is comparable to or higher than some previous reports, most notably the study by Lia Meilawati (2021). In that study, the content was determined to be 1.57 ± 0.03 mg/g for juice and 2.45 ± 0.2 mg/g for ethanol extract. These findings suggest that scopoletin is readily isolated by methanol and ethanol due to its moderately polar nature. Furthermore, the coumarin group is considered one of the essential indicator compounds associated with the biological activity of *M. citrifolia* [9].

The content of compounds such as polyphenols, flavonoids, and scopoletin in *Morinda citrifolia* fruit may vary depending on the developmental stage and ripeness of the fruit. The accumulation of secondary metabolites in plants is often influenced by physiological conditions and environmental factors during growth. Therefore, differences in the maturity stage at the time of harvest may lead to variations in the levels of these bioactive compounds. Further studies investigating these compounds at different ripening stages are necessary to determine the optimal harvesting stage, which would contribute to improving the quality and utilization of Noni as a medicinal plant resource [12].

5. CONCLUSION

The experimental results indicated that the total flavonoid and polyphenol contents reached 25.232 mg QE/g and 6.413 ± 0.112 mg GAE/g of dry extract, respectively, while the scopoletin concentration was 1.636 ± 0.05 mg/g dry extract when extracted with methanol. The quantification of these phyto-chemicals provides a scientific basis for the traditional therapeutic claims of Noni, confirming its potential as a bioactive-rich botanical source. The presence of key constituents, particularly coumarins and flavonoids identified in the Noni fruit samples, provides essential chemical data that support the transition from traditional experience-based use to a more evidence-based approach. These data contribute to the development of quality control criteria and the standardization of herbal materials. In addition, the results provide preliminary information on the chemical characteristics of the investigated Noni samples, which may serve as a reference for further studies, including biological activity evaluation, clinical research, and formulation development. Overall, this study contributes additional scientific data for the investigation and rational utilization of Noni resources in Vietnam, while supporting the sustainable use of plant-derived medicinal resources.

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Xây dựng tiêu chuẩn chất lượng cho dược liệu Nhàu (*Morinda citrifolia* L., họ Rubiaceae) thu hái tại Công ty Mephydica

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TÓM TẮT

Đặt vấn đề: Trái nhàu (Morinda citrifolia) được sử dụng rộng rãi trong y học dân gian với mục đích hỗ trợ sức khỏe; tuy nhiên, các dữ liệu khoa học định lượng về các thành phần hoạt tính vẫn còn hạn chế. Nhiều nghiên cứu cho thấy flavonoid, polyphenol, coumarin và scopoletin là những hợp chất liên quan đến hoạt tính sinh học của trái nhàu, nhưng hàm lượng của chúng phụ thuộc lớn vào nguồn nguyên liệu, độ chín và phương pháp chế biến. Việc thiếu kiểm soát chất lượng và liều dùng có thể ảnh hưởng đến hiệu quả và độ an toàn khi sử dụng. Mục tiêu nghiên cứu: Nghiên cứu này nhằm tiến hành sàng lọc hóa thực vật sơ bộ, sắc ký lớp mỏng (TLC) và định lượng flavonoid toàn phần, polyphenol toàn phần và scopoletin trong trái nhàu. Đối tượng và phương pháp nghiên cứu: Trái nhàu được thu thập từ Công ty Trách nhiệm hữu hạn Mephydica. Thành phần hóa học được đánh giá thông qua sàng lọc hóa thực vật sơ bộ và TLC. Hàm lượng flavonoid toàn phần (TFC) và polyphenol toàn phần (TPC) được xác định lần lượt bằng phương pháp tạo phức với nhôm clorid và thuốc

thử Folin-Ciocalteu. Scopoletin được định lượng bằng phương pháp quang phổ UV-Vis. Kết quả: Kết quả sàng lọc hóa thực vật cho thấy sự hiện diện của nhiều hợp chất có hoạt tính sinh học như coumarin, saponin và các chất khử. Phân tích TLC ghi nhận sự xuất hiện của vết sắc ký tương ứng với chất chuẩn scopoletin. Hàm lượng flavonoid toàn phần trong cao chiết methanol là 25.232 ± 0,023 mg QE/g cao khô; hàm lượng polyphenol toàn phần là 6.413 ± 0.112 mg GAE/g cao khô. Hàm lượng scopoletin được xác định là 1.636 ± 0.05 mg/g cao khô. Kết luận: Nghiên cứu đã cung cấp các dữ liệu định lượng về flavonoid, polyphenol và scopoletin trong cao chiết methanol của trái nhàu, góp phần củng cố cơ sở khoa học cho việc sử dụng theo kinh nghiệm dân gian, đồng thời hỗ trợ chuẩn hóa chất lượng và định hướng cho các nghiên cứu ứng dụng chuyên sâu trong tương lai.

Từ khóa: *Morinda citrifolia, flavonoid tổng, polyphenol tổng, scopoletin, UV-Vis*

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