

Microscopic and physicochemical evaluation of *Ruta angustifolia* leaves

Tutik Sri Wahyuni,^{1,2} Ni'matul Khoiriyah,³ Lidya Tumewu,² Wiwied Ekasari,^{1,2} Achmad Fuad,^{1,2} Aty Widayawaruyanti^{1,2}

¹Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia; ²Center of Natural Product Medicine Research and Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia; ³Undergraduate student, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

Abstract

Background: There are thousands of species of known medicinal plants in the world. *Ruta angustifolia* L. has been widely used in traditional medication for jaundice and liver disease. Previous studies have shown that *R. angustifolia* leaves can inhibit the hepatitis C virus in Huh7 culture cells, reduce the value of NS3 protein, and possess a synergistic effect in combination with antiviral drugs. Therefore, this plant is potential to be developed as a drug candidate. Characteristics of plants including microscopic, physicochemical properties, and phytochemical profiles are necessary information to ensure the quality of raw material in drug development.

ment.

Objective: This study was carried out to examine the microscopic and physicochemical including the standardized parameter of *R. angustifolia* leaves to fulfil the quality raw materials as traditional medicine.

Methods: Simplicia of *R. angustifolia* leaves obtained from Jombang, East Java, were observed under a microscope and determined its physicochemical properties referred to the Materia Medica Indonesia V. The TLC and HPLC profiles of extract were determined as well.

Results: Microscopic analysis were conducted by transfection sections and the presence of epidermis cells, palisade, mesophyll with stomata, and Ca-oxalate crystal were found. The standard parameter obtained value of loss of drying, extractive value in water and ethanol, and ash value. The TLC and HPLC profiles of leaves extract demonstrated to contain with flavonoid, terpenoids, and alkaloids.

Conclusion: *Ruta angustifolia* obtained from Jombang, east Java, has a specific character in microscopic analysis. The physicochemical properties analysis of *R. angustifolia* leaves as a raw material met the requirements according to Materia Medica Indonesia V.

Correspondence: Tutik Sri Wahyuni, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, 60115, Surabaya, Indonesia.

Tel.: +62.81241437599

E-mail: tutik-s-w@ff.unair.ac.id

Key words: *Ruta angustifolia*, Medicinal plants, Raw material, Infectious disease.

Acknowledgments: The author would like to thank the Ministry of Research and Technology for funding through the Penelitian Dasar scheme with grant number 4/AMD/E1/KP.PTNBH/2020.

Contributions: TSW; design study, drafting, funding, manuscript editing; NK, collecting and analyzing data; LT, data collection and manuscript editing; WE, data analysis and manuscript editing; AF, manuscript editing; AW, analysis of phytochemistry and manuscript editing. All the authors approved the final version to be published.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: None.

Availability of data and material: Data and materials are available by the authors.

Informed consent: The manuscript does not contain any individual person's data in any form.

Received for publication: 22 December 2022.

Revision received: 4 January 2023.

Accepted for publication: 20 January 2023.

This work is licensed under a Creative Commons Attribution NonCommercial 4.0 License (CC BY-NC 4.0).

©Copyright: the Author(s), 2023

Journal of Public Health in Africa 2023; 14(s1):2520

doi:10.4081/jphia.2023.2520

Introduction

Thousands of plant species were known as medicinal plants in the world. Approximately 30,000 species of medicinal plants were allegedly located in Indonesia. From the abundant medicinal plants present, only 1,200 types of plants have been used as raw materials for herbal medicines.^{1,2} Medicinal plants can be used as a source of drug candidates for infection with the hepatitis C virus (HCV).³ Apart from having active chemical components, medicinal plants are also exhibited as inhibitors in the replication of various DNA and RNA viruses.⁴ Various treatments have traditionally been used as a therapy for HCV infection treatment, and some of these treatments have gone through various tests including clinical trials and some have theoretical efficacy.⁵ It was also reported several medicinal plants possess anti-HCV activities with various mechanisms.^{3,6,7}

Ruta angustifolia L. is a plant of Rutaceae and is locally known as inggu. Ruta has been reported to be used as various traditional medicine including antiseptic, anti-inflammatory, pain relief, respiratory tract treatment, nervous system, and musculoskeletal.⁸ Meanwhile, it was used as a traditional remedy for jaundice and liver disease in Indonesia.⁹

Ruta angustifolia L. plant contains alkaloids, coumarin, and flavonoids. Our previous studies have isolated chalepin and pseudane IX which exhibited strong antiHCV activity.¹⁰ *Ruta angustifolia* extract was able to decrease the protein value of NS3 HCV and work at the post-entry step. A synergistic effect was shown when *R. angustifolia* extract was combined with the antiviral HCV

drugs.¹¹ Moreover, it was also reported that chalepin and rutamarin can significantly inhibit the growth of cancer cells.¹² Those suggested that *R. angustifolia* L. is the potential to be developed into a product of traditional medicine, including standardized herbal medicine. In its development, raw materials products had certain requirements which are important for their pharmacological and safe effects. Thus, to ensure the constancy and clarity of the ingredients contained in preparations from natural raw materials so that it is expected to have a fixed pharmacological effect from time to time, evaluation of drug raw materials following standard parameters is needed. The raw material must meet the requirements listed in the monograph published by the Ministry of Health of the Republic of Indonesia, namely *Materia Medica Indonesia* and *Indonesian Herbal Pharmacopoeia*. These requirements include raw material identity, microscopic test, organoleptic, water-soluble extract, ethanol-soluble extract, loss on drying, total ash content, acid-insoluble ash content, water-soluble ash content, and determination of certain chemicals.

Therefore, this research aims to conduct the standard parameters of inggu leaves (*R. angustifolia* L.) as the raw material in the development of traditional medicines through microscopic and physicochemical properties evaluation to guarantee the quality of the medicinal product.

Materials and Methods

Materials

This study was inggu leaves that were cultivated in Jombang, East Java, Indonesia. The plant was determined by a botanist of *Materia Medica Indonesia* with specimen number 074/319A/102.7/2019. The plants were collected at the age of 10-12 months. It was sorted, cleaned, and dried at room temperature and protected from direct sunlight for 3 weeks. The dried simplicia is made into a powder by grinding and followed by further analysis.

Microscopic analyses

The test is carried out under a light microscope. The magnification is adjusted to the need of the study. The anatomy of the inggu leaves and the fragments present in the leaves powder were examined and compared to the reference. Water was used as media and chloral hydrate was used as a reagent to dissolve the cytoplasm.

Physicochemical properties

Physicochemical properties were evaluated including the percentage of substance dissolve in water and ethanol, total ash content, acid-insoluble ash, and loss of drying.¹³

Water-soluble extract determination

Five g powder of inggu was macerated with 100 mL of saturated water, shaken repeatedly for the first 6 hours, and further macerate during 18 hours. A number of 20 mL of filtrate was evaporated, dried in a shallow dish that has been heated at 105°C several times to obtain the constant balance. The percentage of the water-soluble extract was calculated compared to the reference.

Ethanol-soluble extract determination

Five g of inggu dried powder was macerated with 100 mL of 95% ethanol in flask, and shaken repeatedly for the first 6 hours and 18 hours. The 20 mL of the filtrate was evaporated to obtain the dried material in a shallow dish at 105°C and tapped, heated in the same temperature until the constant weight. The value in % of ethanol soluble extract was determined.

Determination of dry loss

As much as one to two g of simplicia is placed in a shallow, weighed bottle that has been preheated at fixing temperature and set as tare. Flatten the ingredients in the weighing bottle, shaking until got a layer about 5 to 10 mm thick. It was heated at 105°C in the oven for one hour and following by cooling in desiccators. It was dried chamber, opened the lid, and dried at a fixed temperature to a fixed weight. While drying, the bottle was closed and started cooling in the desiccator to room temperature. Loss in weight was called as moisture content that was used to calculate its percentage by comparing to initial weight of simplicia.

Total ash determination

The total ash was conducted by weighing two g of inggu powder that has been mashed and put into crushed porcelain crucibles. It was incinerated slowly at 600 °C until the charcoal runs out, cool, and weighed. Additional hot water was necessary If the charcoal cannot be removed. It was stired and filtered through ash-free filter paper. It was spread in the crucible with the filter, steamed, and heated it until the weight remains. The percentage of total ash was calculated compared to the weight of the powder.

Acid-insoluble ash determination

The obtained ash from the total ash calculation was washed with 25 mL of dilute hydrochloric acid (2 N) and boiled for five minutes. Gathering the insoluble part in acid which were filtered through ash-free filter paper. It was washed with hot water and sprinkled in crushers until the weight remains. Ash content which is insoluble in acid is calculated against the weight of the crude material.

Determination of water-soluble ash

In a crucible containing total ash, add 25 mL of water and boil for five minutes. Insoluble substances are filtered using ash-free filter paper. Washed with hot water and incubated in a crucible for 15 minutes at a temperature of no more than 450°C, open the lid of the porcelain crucible. Spread to constant weight. Water-soluble ash content is calculated compared to the weight of the crude material.

Table 1. Physicochemical characteristic of *R. angustifolia* leaves.

Parameters	Value* (% w/w)	Requirement (materia medica indonesia V) (%)
Water-soluble extract	27.14±0.47	Not less than 28
Ethanol-soluble extract	20.17±0.43	Not less than 8
Total ash	10.75±0.01	Not less than 10
Water-soluble ash	2.67±0.47	No specific parameter
Acid-insoluble ash	0.52±0.05	Not more than 3
Loss on drying	10.02±0.21	Not more than 10

*Mean±SD in triplicate.

Table 2. Determination of heavy metal.

Test	Result	Requirements
Pb	0.12 ppm	≤10 ppm
Cd	0.01 ppm	≤0.3 ppm
Hg	0.00013 ppm	≤0.5 ppm
As	0.00002 ppm	≤5 ppm

Determination of heavy metal

The presence of heavy metal (lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As)) were determined by Atomic Absorbance Spectrophotometer (AAS). Five g samples were digested with suitable reagents such as 9 mL HNO₃ and 3 mL H₂O₂ and heated at 180°C for 25 min. After this destruction process, the sample was diluted and analyzed. A calibration curve is made by absorbing the standard series which has been corrected to the blank, and the metal content. The metal content in the sample is calculated.

Phytochemical profile with thin layer chromatography and high-performance liquid chromatography

The phytochemical screening was conducted by TLC using silica gel GF₂₅₄ as a stationary phase, hexane-acetone (3/2 v/v) as a mobile phase, and spray reagent FeCl₃, borate citrate, and dragendorff for identification of polyphenol, flavonoid and alkaloid compounds, respectively. Meanwhile, terpenoid identification using silica gel GF₂₅₄ as a stationary phase, hexane-ethyl acetate (7/3 v/v)

as a mobile phase, and spray reagent anisaldehyde sulphate.

Ruta angustifolia extract was subjected to HPLC to determine the peaks' chromatogram profile and UV spectra. HPLC Shimadzu was used for this experiment, completed with RP-18 column (Merck, 4.6x250 mm, 5 μm), using acetonitrile-water (80%-20% v/v) as mobile phase, flowrate 0.5 mL/min. The extract (1,000 ppm) was injected with a volume of 40 μL and a chromatogram was observed at UV 254 nm for 30 minutes.

Results

Microscopic analysis

The plant *R. angustifolia* showed as an herb with small leaves (Figure 1). This plant was found less than 1 meter high, the leaves are green, oblong to an oval and smooth surface. A microscopic profile of the transection section of *R. angustifolia* leaves was shown to have an epidermis, upper and lower palisade tissue, spongy tissue,



Figure 1. *Ruta angustifolia* plants. A) parts of the plant as a whole; B) part of the leaves.

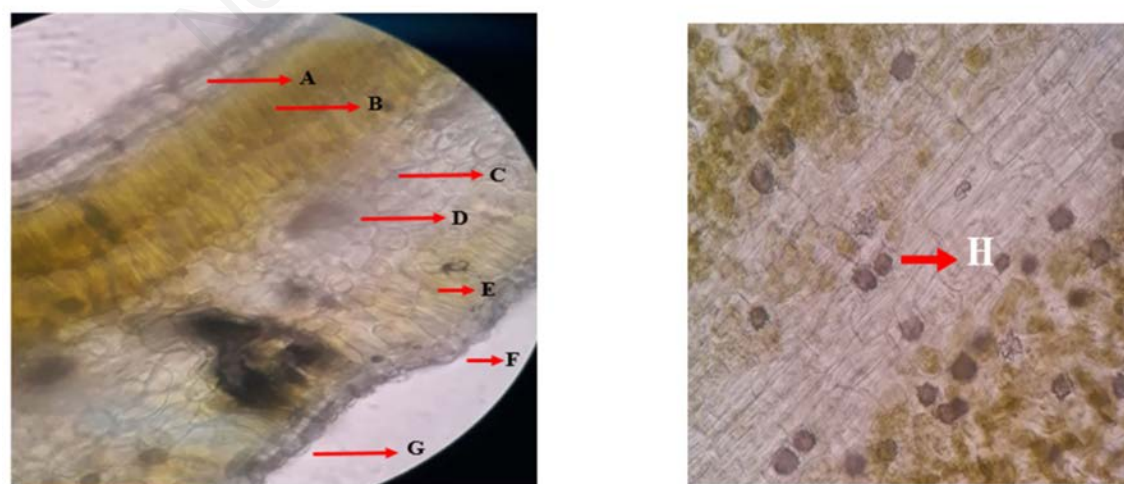


Figure 2. Microscopic analysis on the simplicia powder of *R. angustifolia* leaves. A: Upper epidermis. B: Upper palisade tissue. C: Spongy tissue. D: Lysigen gland. E: Lower palisade tissue. F: Stomata. G: Lower epidermis. H: Calcium oxalate rosette-shaped crystal.

lysigen gland, stomata, and calcium oxalate rosette-shaped crystal (Figure 2). Further evaluation of its leaves powder by observing under the microscope found tissue of palisade, mesophyll, epidermis, stomata, crystal, oil gland, and tissue vessel (Figure 3).

Physicochemical properties

Physicochemical properties determination of *R. angustifolia* leaves was obtained from the parameter value of water-soluble extract, ethanol-soluble extract, total ash, water-soluble ash, and acid-insoluble ash. The water-soluble extract value obtained was $27.14 \pm 0.47\%$, which indicated the amount of polar substance of the plant which dissolve in the water. While the ethanol-soluble was $20.17 \pm 0.43\%$, indicating the extracted components of the plant in ethanol. Total ash is important to evaluate the amount of external and internal minerals in simplicia. The total value of ash obtained was $10.75 \pm 0.01\%$. The ash was dissolved in water and water-soluble ash was $2.67 \pm 0.47\%$. It was also dissolved in acid to determine the acid-insoluble ash, the value was $0.52 \pm 0.05\%$. The acid-insoluble parameter indicated the impurities such as silicate in the material. Another tested parameter was the loss of drying that measured the value of the compound due to the process of drying

and it was revealed of $10.02 \pm 0.21\%$. Those results were relatively within the limit of *Materia Medica Indonesia V* (Table 1).

Heavy metal evaluation

Heavy metals were external contamination that need to be measured to guarantee the safety of the material. Heavy metal contamination testing aims to determine the value of metal content including Pb, Cd, Hg, and As in the simplicia of *R. angustifolia*. Those metals contributed dangerously to human health. The value of Pb, Cd, Hg, and As were 0.12 ppm, 0.01 ppm, 0.00013 ppm, and 0.00002 ppm, respectively (Table 2).

TLC and HPLC profile

The phytochemical evaluations of leaves extract were conducted by TLC and HPLC. Based on the TLC profile, it was found that leaves of *R. angustifolia* contain an alkaloid, flavonoid, terpenoid, and polyphenol compounds (Figure 4). While the HPLC profile of the extract detected several peaks at Retention Time (Rt) of 3.9 min, 5.2 min, 6.0 min, 7.4 min, 8.7 min, and 10.5 min. Those peaks were further observed in the UV spectrum to predict the groups of compounds contained in the extract (Figure 5).

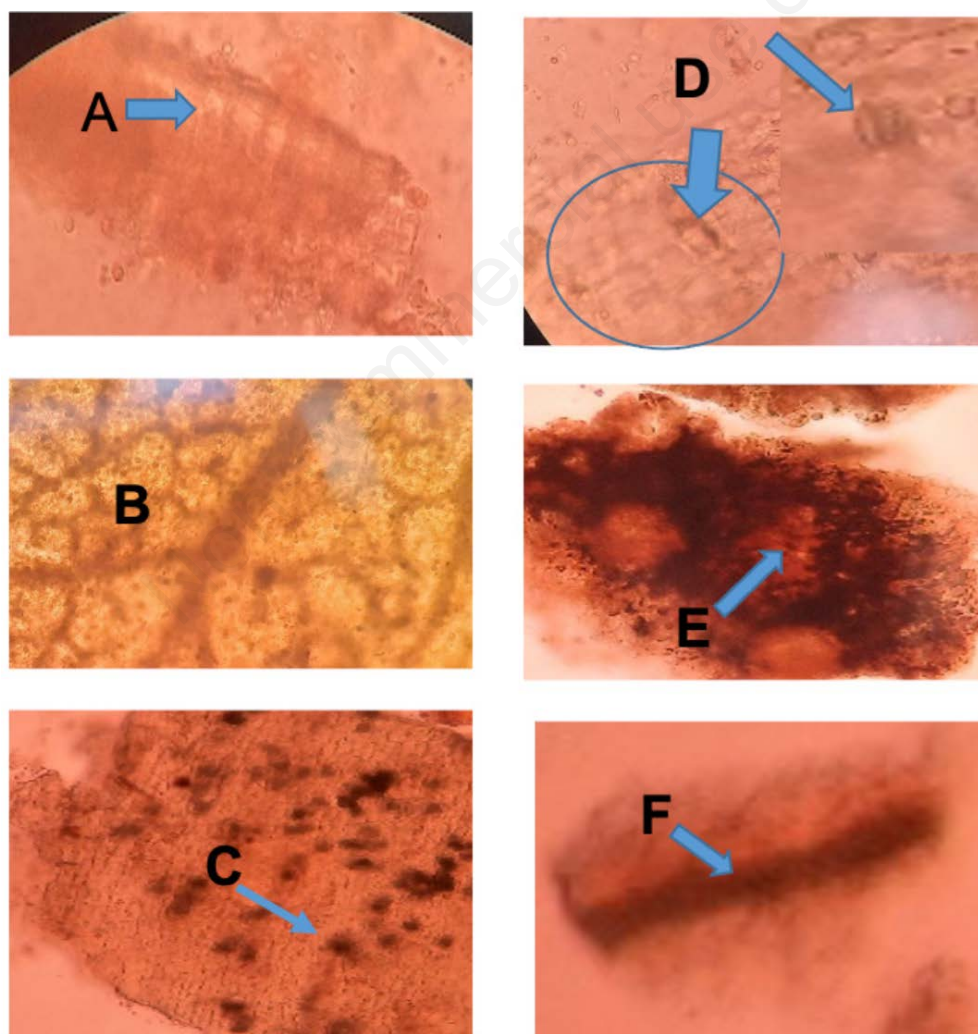


Figure 3. Microscopic profile of *Ruta angustifolia*. A: Palisade; B: Mesophyll; C: Epidermis with crystal; D: Stomata; E: Volatile oil cavity; F: Vessel fragment.

Discussion

Microscopic analysis

Microscopic tests are characteristic elements of anatomy or tissue which identify the types of raw material based on the specific identification fragments.¹⁴ It was found that the upper and lower epidermis consisted of one rectangular cell layer, the upper palisade tissue consisting of two long cylindrical cell layers, the lower palisade tissue consisting of one cylindrical cell layer, and slightly bent long, essential oil glands, spongy tissue consisting of several layers of cells that are irregular in shape and there are many air cavities, idioblasts with calcium oxalate rosette-shaped crystals, vascular bundles, and anomocytic stomata. The fragments found from the observations match the identification fragments listed in the *Materia Medica Indonesia V*, namely the lower epidermis, upper epidermis, calcium oxalate rosette-shaped crystal, and vessel bundles.^{15,16}

Physicochemical properties

Water-soluble extract value is used for evaluating crude drugs

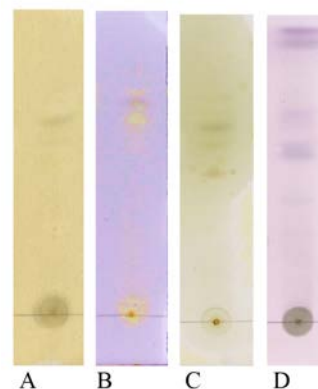


Figure 4. The TLC profile of *R. angustifolia* extract using silica gel GF254 as a stationary phase, hexane-acetone (3/2 v/v) (A-C), and hexane-ethyl acetate (7/3 v/v) (D) as a mobile phase; spray reagent FeCl₃ (A), borate citrate (B), dragendorff (C), and anisaldehyde sulphate (D) for identification of polyphenol, flavonoid, alkaloid, and terpenoid, respectively

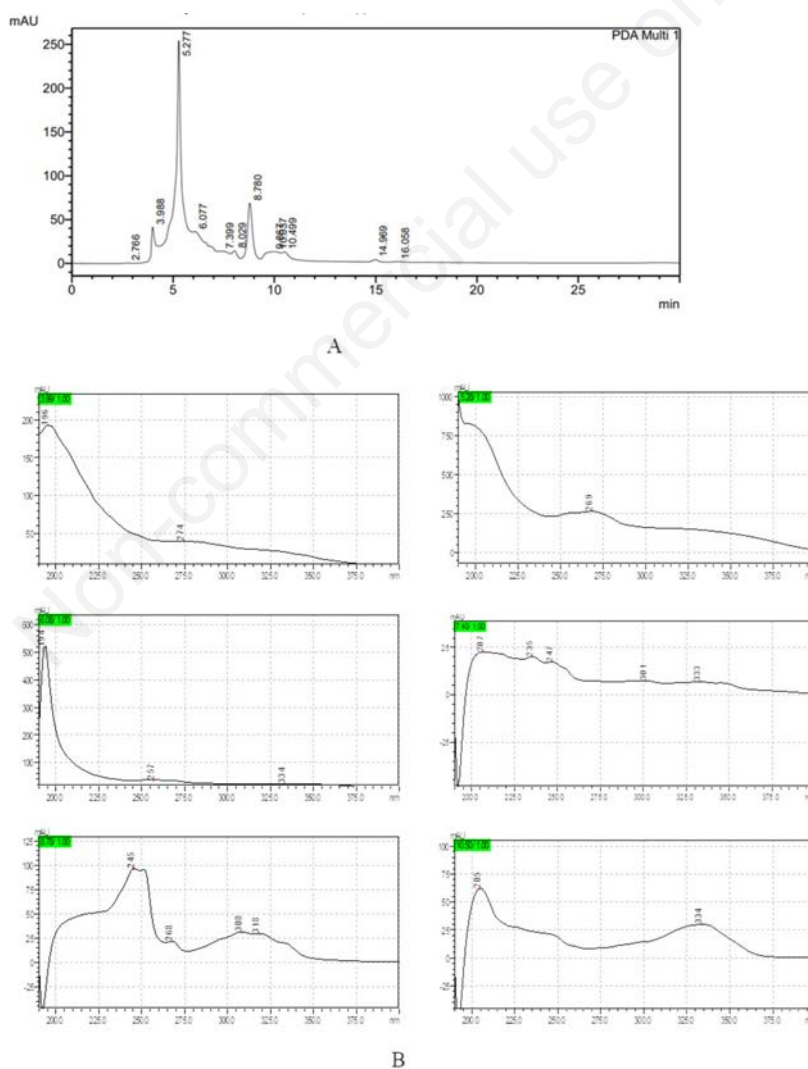


Figure 5. The HPLC profile of *R. angustifolia* extract observed under UV 254 nm using RP-18 column (Merck, 4.6x250 mm, 5 μ m) and acetonitrile-water (80%-20% v/v) as mobile phase with a flow rate of 0.5 mL/min (a), The UV spectra of several peaks at Rt 3.9 min, 5.2 min, 6.0 min, 7.4 min, 8.7 min and 10.5 min (b)

which indicated an approximate measure of their chemical constituents. Water-soluble extract value aims to determine the number of polar compounds that can be extracted by water solvents.¹³ In determining the concentration of water-soluble extract, the result was 27.14% \pm 0.47. The polar compounds found in *Ruta angustifolia* L. include rutin, tannins, and saponins which may be extracted in the water solvent.¹⁷ The soluble extractive of ethanol aims to determine the amount of semi-polar or non-polar compounds that can be extracted using ethanol as a solvent.¹⁸ For the determination of the content of extracts that dissolve in ethanol, the result was 20.17% \pm 0.42, this result met the requirements listed in *Materia Medica Indonesia V*, which is not less than 8%. The semi-polar or non-polar compounds found in the *R. angustifolia* L. plant include essential oils, sterols, triterpenoids, chalepin, γ -fagarine, arborinine, and kokusaginine.¹⁰ Loss on drying provides an overview of the maximum limits on the number of compounds lost during the drying process, which can be lost after heating at 105°C including water and volatile compounds such as essential oils.^{6,13} In the determination of loss on drying, the yield was 10.02 \pm 0.21%. The value obtained is due to the loss on drying for not only water but volatile compounds such as essential oils as well. This can happen because the raw material is still not completely dry.

The total ash content shows the internal and external mineral content in the raw material and extract, if the total ash content value is high it indicates the mineral content in the raw material. In determining the total ash content, the result obtained was 10.75 \pm 0.01%. This result has met the requirements set out in *Materia Medica Indonesia V*, which is not less than 10%. The higher the total ash content, the higher the mineral content in the raw material.^{6,13,19}

Acid-insoluble ash content indicates the amount of mineral content that is insoluble in acid. If the value of acid-insoluble ash content is high, it shows that the raw material and extracts studied contain silicate content from soil or sand, silver metal, lead, and mercury.^{6,17,20} For the determination of the acid-insoluble ash content, the result was 0.52 \pm 0.05%. This result has met the requirements set out in *Materia Medica Indonesia V*, which is not more than 3%.

Heavy metal evaluation

The heavy metal contamination test aims to assure that raw material does not contain certain heavy metals such as Hg, Pb, Cd, and As which exceed the predetermined value because they are harmful (toxic) to health.²⁰ Hg (mercury) can damage the nervous system, digestive system, immune system, kidneys, and lungs. Meanwhile, Pb (lead) and Cd can cause abdominal pain, constipation, headaches, frequent forgetting, and pain in the hands or feet. The high amount can cause anemia, and kidney and brain damage. Moreover, arsenic exposure may cause cancer.^{21,22}

In the determination of heavy metal contamination for Pb, Cd, Hg, and As, the results showed the content was 0.12 ppm, 0.01 ppm, 0.00013 ppm, and 0.00002 ppm, respectively. The results of this determination have met the requirements for raw material, namely for Pb metal \leq 10 ppm, Cd metal \leq 0.3 ppm, Hg metal \leq 0.5 ppm, and metal As \leq 5 ppm.^{19,20}

TLC and HPLC profile

The phytochemistry screening of *R. angustifolia* extract by TLC in this study showed the presents of polyphenols, alkaloids, flavonoids, and terpenoids groups of compounds. The compounds were indicated by yellow spots for polyphenols and flavonoids, orange to brown spots for alkaloids, and violet spots for terpenoids after being sprayed with FeCl₃ and borate citrate, dragendorff, and anisaldehyde sulphate, respectively (Figure 4). Meanwhile, the HPLC chromatogram profile of *R. angustifolia* extract showed

several peaks under 30 min observation (Figure 5A). Each peak was observed with its UV spectra to predict the group of compounds contained in the extract (Figure 5B). The TLC results were in accordance with the UV spectra of HPLC chromatogram peaks detected in the extract. The UV spectra have λ maks at 269 and 274 nm which represent peaks detected at Rt 5.2 min and 3.9 min. These UV spectra possibly represent alkaloids due to the dihydroindole chromophore of alkaloids absorption maximal near about 250 and 300 nm.²³ The main absorption features of this phenolic subfamily are given by an intense absorption band at 280 nm.²⁴ The UV spectra have λ maks at 235 nm which represent peaks detected at Rt 7.4 min. The terpenoid types were reported to show a simple absorption at 235.60 nm. Furthermore, other UV spectra have λ maks at 194, 257, and 334 nm for a peak at Rt 6.0 min; λ maks at 247, 301, and 333 nm for a peak at Rt 7.4 min; λ maks at 245, 268, 308, and 318 nm for a peak at Rt 8.7 min; and λ maks at 205 and 334 nm for a peak at Rt 10.5 min. These four UV spectra possibly represent flavonoid compounds. The typical UV spectra of flavonoids include two absorbance bands. In flavanones and dihydroflavonols, band A lies at 300-330 nm and band B at 277-295 nm range.²⁵ The results suggested that alkaloids, terpenoids, and flavonoids were present in the extract.

Conclusions

The results suggest that *R. angustifolia* leaves simplicia obtained from Jombang, East Java, revealed the specific characteristic and possesses standard parameters according to *Materia Medica Indonesia V* to ensure the quality of the raw material of the drug product.

References

- Sen T, Samanta SK. Medicinal plants, human health, and biodiversity: a broad review. *Adv Biochem Eng Biotechnol* 2015;147:59-110.
- Yacoub K, Cibis K, Risch C. Biodiversity of Medicinal Plants. *Biodiversity, Natural Products and Cancer Treatment* 1-32.
- Wahyuni TS, Utsubo CA, Hotta H. Promising Anti-Hepatitis C Virus Compounds from Natural Resources. *Natural product communications* 2016;11:1193-200.
- Javed T, Ashfaq UA, Riaz S, Rehman S, Riazuddin S. In-vitro antiviral activity of *Solanum nigrum* against Hepatitis C Virus. *Virol J* 2011;8:26.
- Milliman WB, Lamson DW, Brignall MS. Hepatitis C; a retrospective study, literature review, and naturopathic protocol. *Altern Med Rev* 2000;5:355-71.
- Sumbul S, Ahmad MA, Asif M, et al. Physicochemical and phytochemical standardization of berries of *Myrtus communis* Linn. *J Pharm Bioallied Sci* 2012;4:322-6.
- Wahyuni T, Azmi D, Permanasari AA, Adianti M, Tumewu L, Widiandani T, et al. Anti-viral activity of *Phyllanthus niruri* against hepatitis C virus. *Malaysian Applied Biology* 2019;48:105-11.
- Pollio A, De Natale A, Appetiti E, et al. Continuity and change in the Mediterranean medical tradition: *Ruta* spp. (Rutaceae) in Hippocratic medicine and present practices. *J Ethnopharm* 2008;116:469-82.
- Richardson JS, Sethi G, Lee GS, Malek SN. Chalepin: isolated from *Ruta angustifolia* L. Pers induces mitochondrial mediated apoptosis in lung carcinoma cells. *BMC Complement Altern Med* 2016;16:389.

10. Wahyuni TS, Widyawaruyanti A, Lusida MI, et al. Inhibition of hepatitis C virus replication by chalepin and pseudane IX isolated from *Ruta angustifolia* leaves. *Fitoterapia* 2014;99:276-83.
11. Wahyuni TS, Mahfud H, Permatasari AA, et al. Synergistic anti-hepatitis C virus activity of *Ruta angustifolia* extract with NS3 protein inhibitor. *J basic clin physiol and pharmacol* 2019.
12. Fakai MI, Karsani S.A, Malek SNA. Chalepin And Rutamarin Isolated from *Ruta angustifolia* Inhibit Cell Growth in Selected Cancer Cell Lines (MCF7, MDA-MB-231, HT29, and HCT116). *JISTM* 2017;2:10.
13. Mukherjee PK. Chapter 3 - Quality Evaluation of Herbal Medicines: Challenges and Opportunities. *Quality Control Evaluation of Herbal Drugs*. Elsevier 2019;53-77.
14. Nazish I, Ali M, Mir SR, et al. Application of Microscopy in Authentication of Unani Traditional Plant *Ruta graveolens* 2010.
15. Mukherjee PK. Chapter 5 - Morphological and Microscopical Evaluations. In: Mukherjee PK, editor. *Quality Control and Evaluation of Herbal Drugs*: Elsevier 2019;151-93.
16. Mattummal R, Divya K, Kn SK. Comparison of the Leaf Drug *Ruta graveolens* and Its Substitute *Ruta chalepensis*. *Intern J Res Ayurveda Pharma* 2018;9:100-10.
17. Alotaibi S, Saleem M, Al-humaidi J. Phytochemical contents and Biological Evaluation of *Ruta chalepensis* L growing in Saudi Arabia. *Saudi Pharma J*. 2018;26.
18. Tutik Sri W, Adita Ayu P, Lidya T, et al. Qualitative and Quantitative Analysis of 70% Ethanol Extract from *Ruta angustifolia* for Developing Anti-Hepatitis C Agents. *Pharmacognosy J*. 2021;13.
19. Organization WHO. *Quality control methods for medicinal plant materials*. 1998.
20. Mukherjee PK. Chapter 4 - Qualitative Analysis for Evaluation of Herbal Drugs. In: Mukherjee PK, editor. *Quality Control and Evaluation of Herbal Drugs*: Elsevier 2019;79-149.
21. Bernard A. Cadmium & its adverse effects on human health. *Indian J Med Res* 2008;128:557-64.
22. Hong YS, Song KH, Chung JY. Health effects of chronic arsenic exposure. *J Prev Med Public Health* 2014;47:245-52.
23. Govindachari TR, Rajappa S. Ultraviolet absorption spectra of dihydroindole alkaloids. *Proceedings of the Indian Academy of Sciences - Section A* 1960;51:319-21.
24. Wessel JR. An adaptive orienting theory of error processing. *Psychophysiol* 2018;55.
25. Tsimogiannis D, Samiotaki M, Panayotou G, Oreopoulou V. Characterization of flavonoid subgroups and hydroxy substitution by HPLC-MS/MS. *Molecules* 2007;12:593-606.

Non-commercial use only