Jurnal Riset Kimia

ISSN (print) : 1978-628X
ISSN (online) : 2476-8960

Identification of Secondary Metabolites and FT-IR Analysis of Getih-Getihan Fruit Extract (*Rivina humilis* L.)

Mariyam Mariyam[a]*, Yulistia Anggraini[a], and Tati Suhartati[b]

[a]Department of Chemistry, Faculty of Science, Institut Teknologi Sumatera, South Lampung, Indonesia

[b]Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Indonesia

Corresponding Author:
Mariyam Mariyam
mariyam@ki.itera.ac.id

Received: October 2022
Accepted: February 2023
Published: March 2023

Abstract

Getih-getihan (*Rivina humilis* L.) plants can be used as antibacterial, antioxidant and natural pesticides. Most of the biological activities of natural products originated from secondary metabolites contained therein. Studies have shown the effects of leaves, branches, and fruits extract of *R. humilis* towards biological activities. However, identifying the phytochemical compounds of *R. humilis* L. fruit is less discussed. Here we proposed research on the identification of secondary metabolite compounds of *R. humilis* L. fruit extract using phytochemical screening tests and spectroscopic method. The extraction of *R. humilis* L. fruit was proceeded by maceration method using methanol solvent. The series of phytochemical screening tests signified the presence of alkaloids, terpenoids, tannins and flavonoids. Furthermore, we applied FT-IR analysis to confirm the existence of functional groups in the secondary metabolite compounds. A broad absorption band showed the hydroxyl groups (O-H) at 3265 cm^{-1}. The sharp band at 1632 cm^{-1} exhibited the C=C stretching band. The presence of C-N (stretching) was signified by the absorption band at 1237 cm^{-1}, while the C-H bond in CH_3 terminals (alkanes, alkyl group) was exhibited at 1401 cm^{-1}. All the functional groups confirmed in the FT-IR analysis corroborated the phytochemical test results.

Keywords: *Rivina humilis L., secondary metabolites, phytochemical, FT-IR*

Introduction

The exploration of natural products on their functions as therapeutic agents and traditional herbal medicines in overcoming various diseases has become a great interest in discovering and developing more effective drugs. Medicinal plants hold a unique role as the primary source of new medicines that can be used as an alternative to synthetic drugs. More than 50% of modern drugs come from nature or its derivation[1]. Based on World Health Organization (WHO) data, 80% of the world's population, which is dominated by developing countries, depends upon herbal plants for their essential well-being care needs.

Herbal plants have been used for a long time as medicine, food, and for various daily needs. Recently, there has been a significant increase in the discovery of new antibacterial compounds due to the high rate of infections against antibiotic-resistant microorganisms[2]. Herbal plants often exhibit various biological activities such as anti-inflammatory, antibacterial, and antifungal properties[3],[4]. The foremost capable and promising components of plants are their secondary metabolites that contribute to acting as therapeutics. The active metabolites like phytochemicals from medicinal plants were under investigation for the advancement of novel and successful biodegradable drugs as an option to the ineffective modern pharmaceuticals[1]. The content of chemical compounds of secondary metabolites in natural products has a tremendous function in the development of new types of antibiotic drugs. Some secondary metabolite compounds generally found in plants are alkaloids, terpenoids, flavonoids, tannins, phenols, steroids and saponins[5].

R. humilis L. belongs to the family *Phytolaccaceae*, is known to have medicinal properties and lives in colonies on black soil types[6]. In past studies, the fruit extract of *R. humilis* L. was reported to have antioxidant and anticancer properties due to high betacyanins and betaxanthins pigments[7],[8]. Secondary metabolites of *R. humilis* L. leaves, such as alkaloids, flavonoids, tannins, and terpenoids, showed a Lethal Concentration (LC50) value of 1.415% against *Spodoptera litura* F and can be utilized as a natural pesticide[9]. Moreover, the isolation of betanin compounds (red and dark red pigments) from leaves and fruits of *R. humilis* is known to have antioxidant properties, inhibiting liver toxicity, which was tested pre-clinically[10]. Estimation of secondary metabolites constituent in *R. humilis* fruit is the primary step in determining the properties of the *R. humilis* plant as antimicrobial, antioxidant, anti-inflammatory and anticancer. Our previous study, it was reported that *R.humilis* L. fruit extract possessed antibacterial efficacy against *E. coli* and *K. pneumoniae*[11]. However, the study of its phytochemical contents has not been reported. Hence, investigating these phytoconstituents are essential and would assist in deciding their different biological evaluation. The presence of these secondary metabolites can be determined through phytochemical screening and spectroscopic methods. The combination of these methods can be used to estimate the presence of the constituents in plants. Therefore, in this study, phytochemical screening and FT-IR analysis are carried out to determine the secondary metabolites of the extract of *R. humilis* L. fruit.

Experimental

Materials

R. humilis L. fruit was obtained from the Kedaton district, Bandar Lampung city, Indonesia (latitude: -5.394535, longitude: 105.262679). Chemicals used in this research are methanol (Merck), Mayer reagent, acetic acid glacial, H_2SO_4, Mg powder, HCl, KOH, $FeCl_3$ and distilled water. All chemicals were analytical grade.

Instruments

The instrument used in this research was *Agilent/Cary 630 FTIR* to determine the functional groups contained in the extract.

Methods

Preparation of Plant Material

R. humilis L. fruit (88.609 g) was dried in an oven for 6 hours at 30°C and crushed using a mortar pestle. The extraction was undertaken by the maceration method using methanol solvent only due to the polar properties of the fruit sample [7]. The sample was immersed in methanol solvent by the ratio of sample and the solvent was 1: 4, then stirred for a few minutes and kept in a dark place for 24 hours. The extract was filtered using filter paper, and the maceration process of the sample residue was repeated 7 times. The collected extract was evaporated by using a rotary evaporator. The crude extract obtained was 5.204 g.

DOI: https://doi.org/10.25077/jrk.v14i1.565

Phytochemical Screening

The content of the secondary metabolites in the crude extract was identified through a series of tests following the standard phytochemical test [12]. This phytochemical screening aimed to determine the presence of alkaloids, steroids, terpenoids, flavonoids, saponin and tannin in the fruit extract of *R. humilis* L.

Test of Alkaloids

0.5 ml of the extract was dropped with chloroform, and 5 drops of Mayer reagent were added. A yellowish-white precipitate indicates the presence of alkaloids.

Test for Steroids and Terpenoids

0.5 ml of glacial acetic acid and 0.5 ml of H_2SO_4 were added to 0.5 ml of the crude extract. The presence of terpenoids was indicated with a brownish-red colour change, while the presence of steroids was designated with a blue/purple colour.

Test for Flavonoids

A pinch of magnesium powder was added to 0.5 ml of the crude extract was 0.5 ml. Then 5 drops of concentrated HCl were added. The red or orange colours and foam formation indicate the presence of flavonoids.

Test for Saponin

The crude extract was dissolved in 5 ml of distilled water. Then 10 drops of KOH were added and heated in a water bath at 50°C for 5 minutes, then shaken for 15 minutes. The formation of a 1 cm foam layer indicates the presence of saponin.

Test for Tannin

Three drops of 5% (w/v) $FeCl_3$ were poured into 1 ml of the crude extract. The dark blue colour is indicated the existence of tannins in the sample.

Results and Disscusion

Plant/Sample Determination

The plants were collected from the Kedaton district, Bandar Lampung city, Indonesia and identified/determined at the Herbarium Bogoriense, Botany Division of the Biology Research Center, Indonesian Institute of Sciences, Bogor-Indonesia. The result of plant determination is presented in Table 1.

Preparation and Extraction of The Sample

The sample drying process in the oven for 6 h aimed to reduce the moisture content and decrease the enzymatic reactions to avoid the sample rot. The grinding process was carried out to widen the sample's surface area to increase the interaction between the sample and solvent (methanol). Extraction of *R. humilis* L. fruit was done by maceration method using methanol solvent to dissolve the active compound due to predominantly polar compounds contained in the sample. During immersion of the sample into the solvent, the plasmolysis process occurred and let the sample's cell membrane release, causing the cell wall damage due to the pressure difference between the outside and inside the cell. This process caused the active compounds in the cytoplasm to dissolve in the methanol solvent. The active compounds of the sample will be extracted during the maceration process, depending on the immersion duration. Maceration is the most suitable method for extracting chemical compounds from thermolabile plants.

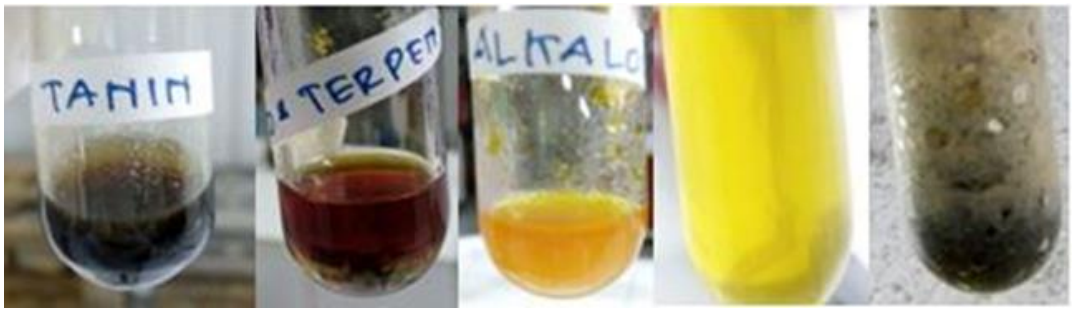
The solvent suitability for the extraction process affects the effectiveness of the expected result by concerning the solubility of the sample in the solvent[13]. The extract was evaporated using a rotary evaporator to reach a thick crude extract. The crude extract obtained was 5.204 grams (Table 2.). *R. humilis L.* fruit used in this study was not completely dry and still contained high water content due to only air-drying method used in sample preparation.

Table 1. Plant determination

No.	Species	Family
1.	*Rivina humilis* L.	Phytolaccaceae

Table 2. % Yield of *R. humilis* L. crude extract

Sample	Colour	Weight of crude extract (g)	% Yield of Crude Extract
R. humilis L. fruit extract	Orange reddish	5.204	0.52



(a) (b) (c) (d) (e)

Figure 1. The results of the phytochemical screening test of *R. humilis* L. fruit extract (a) tannin (b) terpenoids/steroids (c) alkaloids (d) saponin (e) flavonoids

The high-temperature drying treatment will damage the active compounds in the sample; hence the appropriate way to get dried *R. humilis* L. fruits is by using the air-drying method or freeze-drying instrument. The calculation of % yield is low due to the wet fruit sample used.

Identification of Secondary Metabolites Compounds of *R. humilis* L. fruit extract

The secondary metabolite compounds in the extract of *R. humilis* L. fruits were analyzed through phytochemical screening tests. The test showed that the crude extract of *R. humilis* L. fruit contained bioactive compounds, such as terpenoids, alkaloids, flavonoids and tannins (Table 3.). As reported in our previous work[11], these bioactive compounds were assumed responsible for their antibacterial activities. Kavita et.al., (2019)[14] reported that fruit extract of R. humilis L. contains around 18 functional groups such as alkanes, phenol, nitro, primary/secondary amines, aromatic, aliphatic amines, etc. The GC-MS analysis showed 20 compounds with their biological activities[14].

 DOI: https://doi.org/10.25077/jrk.v14i1.565

Table 3. Identification of secondary metabolite compounds results.

Series of Test	Visual Observation (Colour)	Results
Tannin	Bluish black	(+)
Terpenoids and Steroids	Brownish red	(+) Terpenoids
		(-) Steroids
Alkaloids	Yellowish white precipitate	(+)
Saponin	No foam	(-)
Flavonoids	Orange colour with foam formation	(+)

FT-IR Analysis

FT-IR analysis was performed to determine the functional group absorption bands of chemical compounds in the sample. The FT-IR spectrum of *R. humilis* L. fruit extract can be seen in Figure 2. The interpretation of the FTIR spectral data shows that the fruit extract of *R. humilis* L. has a hydroxyl group in the presence of O-H and N-H stretching vibrations that extend to the area of around 3265 cm^{-1}[14]. A strong absorption band at 1632 cm^{-1} indicates the presence of a C=C (stretching) functional group from the carbon double-bond compound, which is generally present in terpenoid or flavonoids compounds[15]. The absorption area of 1401 cm^{-1} is the bending vibration of the C-H alkyl group (-CH_3 terminal).

The low absorption band in the 1237 cm^{-1} region indicates the presence of C-N stretching vibrations, estimated to originate from alkaloids in the extract[14]. This assumption is completed and supported by the phytochemical test observation using Mayer's reagent, which showed a positive test result. The sharp absorption band at 1013 cm^{-1} indicates the C-H group predicted as hydrocarbon vinyl group compounds which are strengthened by the presence of the C=C stretching absorption band at 1632.6 cm^{-1} in the previous explanation. The weak band at 2124 cm^{-1} is assumed as the C=O absorption band from CO_2 molecules in the air.

The qualitative test and FTIR results identified secondary metabolites in *R. humilis* L. fruit (terpenoids, alkaloids, flavonoids, and tannins) so that these compounds seemingly cause its antibacterial activities. In plants, these compounds act as a defense mechanism to avoid any threats in their surroundings, also used by humans as raw materials for medicine and antioxidants.

Most terpenoids are in liquid forms, have an odor and quickly evaporate. The structure of terpenoids is allyl cyclic, where some are unsaturated compounds with one or more double bonds. Consequently, this compound may easily undergo addition reactions with hydrogen, halogens and acids. This compound possesses antibacterial activity by reacting with transmembrane protein on the outer membrane of the bacterial cell wall and forming strong polymeric bonds to damage the membrane[16]. This damage reduces the bacterial cell wall's permeability, resulting in a bacterial cell lacking nutrients and inhibiting bacterial growth. This mechanism may lead to bacterial death.

Table 4. Functional groups and frequencies of crude extract of *R. humilis* L. fruit.

Wave Number (cm^{-1})	Predicted Compounds
3265.1	O-H stretching
1632.6	Terpenoids, flavonoids (C=C stretching)
1401.5	Alkane, alkyl (C-H bending)
1237.5	Alkaloids (C-N stretching)
1013.8	Hydrocarbons (vinyl groups)

Alkaloids are cyclic nitrogen-containing compounds. Many alkaloids possess high biological activities. The antibacterial mechanism of alkaloids disrupts the peptidoglycan components in bacterial cells[17]. This interference causes inhibition of bacterial cell wall growth, advancing to cell death. Furthermore, alkaloids may impede protein synthesis and obstruct metabolism reactions in bacteria.

Flavonoids are phenolic compounds with an aromatic ring containing one or two hydroxy groups (OH). The presence of flavonoids may hinder bacteria cell membrane function by forming complex compounds with extracellular and dissolved proteins[18]. This complexation reaction causes the damage of bacteria cell membrane, followed by the release of intracellular compounds. Another study explained that bacterial inhibition mechanisms on flavonoids are illustrated by disturbing the cell membrane's permeability and preventing the enzymatic reactions involving ATPase and phospholipase [19].

The antibacterial potency of tannin is related to its ability to deactivate enzymes and disturb protein transportation in cells. Tannin can hinder the formation of reverse transcriptase and DNA topoisomerase enzymes to interfere with the growth of bacteria [19].

Conclusions

The phytochemical tests showed positive results for alkaloids, terpenoids, flavonoids, and tannins. The FTIR analysis data completed these evaluations. The presence of the C=C bond and vinyl group exhibited the presence of terpenoids and flavonoids. The alkaloids group was detected by the appearance of the C-N stretching absorption band. The hydroxyl groups, C-H bonds in CH_3 terminals of alkenes and alkyl groups were also detected by FTIR analysis. The results of this study indicated that *R. humilis* L. fruits could possess potential bioactivities. Further investigation into the interaction abilities and the efficacy of the active compounds of *R. humilis* L. fruit is necessary.

Acknowledgments

The author would like to thank the Ministry of Research Technology and Higher Education for providing research grants through the Penelitian Dosen Pemula scheme (contract no. 007/SP2H/LT/DRPM/2018) and Institut Teknologi Sumatera for accommodating the laboratory facilities.

References

1. Anand, U., Jacobo-Herrera, N., Altemimi, A. & Lakhssassi, N., A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. *Metabolites*, 9(11): 258 (2019).
2. Marasini, B. P., Baral, P., Aryal, P., Ghimire, K. R., Neupane, S., Dahal, N., Singh, A., *et al.*, Evaluation of antibacterial activity of some traditionally

 DOI: https://doi.org/10.25077/jrk.v14i1.565

used medicinal plants against human pathogenic bacteria. *BioMed Res. Int.*, **2015**: 1–6 (2015).

3. Asmerom, D., Kalay, T. H. & Tafere, G. G., Antibacterial and antifungal activities of the leaf exudate of *Aloe megalacantha* baker. *Int. J. Microbiol.*, **2020**: 1–6 (2020).
4. Yusoff, S. F., Haron, F. F., Tengku Muda Mohamed, M., Asib, N., Sakimin, S. Z., Abu Kassim, F. & Ismail, S. I., Antifungal activity and phytochemical screening of *Vernonia amygdalina* extract against *Botrytis cinerea* causing gray mold disease on tomato fruits. *Biol.*, **9(9)**: 286 (2020).
5. Jain, C., Khatana, S. & Vijayvergia, R., Bioactivity of secondary metabolites of various plants: a review. *Int. J. Pharm. Sci. Res.*, **10(2)**: 494-504 (2019).
6. Claussen, J. & Slip, D., The status of exotic plants on the Cocos (Keeling) islands, indian ocean. *Environ. Sci.* **17**: 1-16 (2002).
7. Ajaib, M., Zikrea, A., Khan, K. M., Perveen, S., Shah, S. & Karim, A., *Rivina humilis* L: A potential antimicrobial and antioxidant source. *J. Chem. Soc. Pak.*, **35(5)**: 1384-1398 (2013).
8. Khan, M. I., Sri Harsha, P. S. C., Giridhar, P. & Ravishankar, G. A., Pigment identification, nutritional composition, bioactivity, and in vitro cancer cell cytotoxicity of *Rivina humilis* L. berries, potential source of betalains. *LWT*, **47(2)**: 315–323 (2012).
9. Nurhayati, D., Subchan, W. & Prihatin, J., The effect of extract of getih-getihan (*Rivina humilis* L.) on Armyworm (*Spodoptera litura* F.) mortality. *Bioedu.*, **16(1)**: 22-30 (2018).
10. Husein, R. O., Ishmatullah, M. H., Nuralisa, R. A., Amalia, N., Prasetyo, B. A. N. & Zuhrotun, A., Review: isolasi senyawa turunan betalain dan aktivitas farmakologi senyawa betanin dari tanaman *Rivina humilis* L. *Farmaka*, **19(2)**: 109-118 (2021).
11. Mariyam, M., Uji aktivitas antibakteri ekstrak buah *Rivina Humilis* L. terhadap *Klebsiella Pneumoniae* dan *Escherichia Coli*. *JSAT*, **2(1)**: 16–22 (2018).
12. Harborne, J. B., Phytochemical methods—a guide to modern techniques of plant analysis. *Physiol. Plant Pathol.*, **27(2)**: 255–256 (1985).
13. Redha, A., Efek lama maserasi bubuk kopra terhadap rendemen, densitas, dan bilangan asam biodiesel yang dihasilkan dengan metode transesterifikasi in situ. *Jurnal Berlian.*, **10(2)**: 218-224 (2011).
14. Kavitha. & Mary Kensa., GC-MS and FTIR screening of ethanol extract of fruits of *Rivina humilis* L. *Int. J. Botany Stud.*, **6(6)**: 1270–1275 (2021).
15. Brza, M. A., Aziz, S. B., Anuar, H., Ali, F., Dannoun, E. M. A., Mohammed, S. J., Abdulwahid, R. T., *et al.*, Tea from the drinking to the synthesis of metal complexes and fabrication of PVA based polymer composites with controlled optical band gap. *Sci Rep*, **10(1)**: 1-17 (2020).
16. Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M. C. C., Endringer, D. C., Fronza, M. & Scherer, R., Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*, **24(13)**: 1-12 (2019).
17. Mabhiza, D., Chitemerere, T. & Mukanganyama, S., Antibacterial properties of alkaloid extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Int. J. Med Chem.*, **2016**: 1–7 (2016).

18. Karim, Z., Sulistijowati, R. & Yusuf, N., Aktivitas antibakteri ekstrak flavonoid buah mangrove *Sonneratia alba* terhadap bakteri *Vibrio alginolitycus*. JIPK., **6(2)**: 55-60 (2018).

19. Rijayanti, R. P., Luliana, S. & Trianto, H. F., Uji aktivitas antibakteri ekstrak etanol daun mangga *Mangifera foetida* L. terhadap *Staphylococcus aureus* secara in vitro. *Jurnal Mahasiswa PSPD FK Untan*, **1(1)**: 2-18 (2014).

 DOI: https://doi.org/10.25077/jrk.v14i1.565