

Characterization of Secondary Metabolite Compounds from Ethyl Acetate Extract of Japanese Papaya Leaves (*Cnidoscopus Aconitifolius*)

Vitageovani T. Montolalu^a, Rymond J. Rumampuk^{*a}, Emma J. Pongoh^a

^a Department of Chemistry, FMIPA-K, State University of Manado, Tondano, 95618, Indonesia

INFO ARTIKEL

Received : 19 May 2026

Approved : 8 July 2026

Key word:

Cnidoscopus aconitifolius,

Isolation,

Identification,

Flavanone,

NMR

ABSTRACT

Indonesia has abundant biodiversity and great potential as a source for the development of natural organic chemical compounds. One plant that is widely used traditionally is Japanese papaya (*Cnidoscopus aconitifolius*), which is known to have potential as a source of natural chemical compounds because it contains various secondary metabolites with pharmacological activities. This study aims to isolate and identify secondary metabolites from the ethyl acetate fraction of Japanese papaya leaves. Isolation was carried out using the maceration method, and separation and purification were done using gravity column chromatography and thin layer chromatography. The identification of compound structures was carried out using Proton Nuclear Magnetic Resonance (¹H-NMR) and Carbon-13 Nuclear Magnetic Resonance (¹³C-NMR) spectroscopy. The analysis results showed that the obtained isolate is suspected to be a compound in the flavanone group, namely 3',4',5-trihydroxyflavanone.

*e-mail:

rymondrumampuk@unima.ac.id

Introduction

Indonesia is one of the countries with a very high level of biodiversity, so it has a wide variety of plants with various potentials as sources for the development of natural organic chemicals. In addition, some people choose to use plants as traditional medicines, due to the high prices of factory-made medicines that are not matched by the purchasing power of the community [1]. The WHO also estimates that more than 80% of the world's population regularly uses medicinal plants to meet their primary health needs [2]. One plant that contains various types of secondary metabolites and can be used as a medicinal ingredient is Japanese papaya (*Cnidoscopus aconitifolius*) [3].

Cnidoscopus aconitifolius is a plant from the *Euphorbiaceae* family, classified as a shrub that can

reach a height of 6 meters, with finger-shaped leaves 32 cm long and 30 cm wide, and petioles [4]. This plant is used as a food source and as a traditional medicine to treat diabetes, inflammation, and obesity [5,6].

Previous studies have reported that research on *Cnidoscopus aconitifolius* leaves shows the presence of alkaloids, phenols, flavonoids, saponins, tannins, and terpenoids based on phytochemical tests [7]. These compounds can have pharmacological effects, including antimicrobial and antioxidant properties [4]. This finding is reinforced by another study that also reported the presence of steroid compounds in the ethyl acetate fraction [8].

In line with these findings, modern theory explains that secondary metabolites are formed as a result of external stimuli, so that each organism

can produce different metabolites depending on environmental conditions, stress intensity, and plant plasticity [9].

Thus, *Cnidioscolus aconitifolius* species growing in different countries have the potential to produce different secondary metabolites, resulting in varying pharmacological activities. However, chemical research on the ethyl acetate fraction of Japanese papaya leaves growing in Indonesia is still very limited in terms of revealing their secondary metabolite content.

Materials and Methods

Materials

The chemical materials used include Japanese papaya leaves taken from the Maesa UNIMA Housing Complex, South Tondano District, Minahasa Regency, technical solvents including: ethanol, n-hexane, ethyl acetate, butanol, methanol that has been re-distilled, For thin-layer chromatography, the following were used, aluminum silica gel G₆₀F₂₅₄ (E Brand 10554), and as a spot detector, a 10% H₂SO₄ solution in methanol and other materials such as Whatman 42 filter paper, aluminum

Instrumentation

The tools and instruments used include fans, jars, blenders, beakers, measuring cups, funnels, Buchner funnels, pipettes, measuring pipettes, chambers, stirring rods, spatulas, capillary tubes, a set of redistillation equipment, a set of evaporator equipment, a set of gravity column chromatography equipment, vials, analytical balance funnels, ovens, *hot plates*, tweezers, scissors, rulers, and pencils. Analysis uses a set of spectroscopy equipment ¹H-NMR and ¹³C-NMR.

Work Procedure

Sample preparation

Japanese papaya leaves taken from the Maesa UNIMA Housing Complex, South Tondano District, Minahasa Regency were picked, washed, dried, and then blended.

Extraction and Fractionation

Japanese papaya leaf samples were macerated using redistilled 70% ethanol solvent. This produced a filtrate, which was then partitioned sequentially using redistilled n-hexane, ethyl acetate, butanol, and methanol solvents.

Compound Separation

An appropriate amount of ethyl acetate fraction was taken for TLC analysis using ethyl acetate:n-hexane:methanol eluents, with various ratios (5:4:1; 5:3:2; 6:3:1). For column chromatography, an eluent with a ratio of 6:3:1 was used, and for re-column chromatography, an eluent with a ratio of 6:3:1 was still used. The 4 g extract was separated by column chromatography with a silica gel G₆₀F₂₅₄ stationary phase. The results of column chromatography with the same R_f value were combined into fractions.

Compound identification

A total of 20 mg of fraction 8.3 (Isolate F_{8.3}) was selected and its structure was determined using *Nuclear Magnetic Resonance Proton* (¹H-NMR) and *Nuclear Magnetic Resonance Carbon* (¹³C-NMR).

Results and Discussion

Extraction and Fractionation

A sample of 1.9 kg of Japanese papaya leaves (powder) was macerated with redistilled 70% ethanol for 6 x 24 hours, producing 14.3 L of filtrate. The filtrate was then evaporated to produce 155.6 g of concentrated ethanol extract. Fifty grams of the concentrated ethanol extract was then mixed with 50 g of silica gel and ground until homogeneous. After homogenization, the mixture was partitioned sequentially using n-hexane, ethyl acetate, and butanol as solvents

Compound Separation

A 4 g fraction of ethyl acetate was separated using gravity column chromatography with 50 g of silica as the stationary phase and ethyl

acetate:n-hexane:methanol (6:3:1) as the mobile phase. The TLC results were collected in 1 mL vials and 324 eluates were obtained, which were then analyzed by TLC. Each eluate with the same Rf value was combined into a fraction. Seventeen main fractions with the same Rf value were obtained. Next, re-gravity column chromatography was performed on F8 with silica gel as the stationary phase and the same mobile phase. The KKG results were collected in 1 mL vials and 125 eluates were obtained, which were then analyzed using TLC. Each eluate with the same Rf value was combined into a fraction. There were 13 main fractions (F_{8.1}-F_{8.13}) with the same Rf value.

Compound Identification

Fraction F_{8.3} was analyzed using *Proton Nuclear Magnetic Resonance* (¹H-NMR) and *Carbon Nuclear Magnetic Resonance* (¹³C-NMR). Based on the results of ¹H-NMR and ¹³C-NMR spectroscopy analysis tabulated in Table 1, isolate F_{8.3} is supported by several signals that interpret the structure of isolate F_{8.3} as follows, with the proposed structure shown in Figure 2:

Interpretation of proton chemical data in isolate

F_{8.3}

- The presence of six aromatic proton signals at δ H shifts , 5.81; 7.71; 7.61; 7.45; 7.44; 7.21 ppm
- Proton signals appearing at δ H 3.51; 3.65 ppm indicate the presence of CH₂
- The proton signal at a shift of 3.58 ppm indicates a CH group at C-2

Interpretation of carbon chemical data in isolate

F_{8.3}

- There are twelve aromatic carbon signals at shifts of 149.24; 140.26; 129.97; 73.66; 133.67; 125.32; 132.54; 120.14; 114.79; 169.50; 140.6; 120.14 ppm. Of the twelve aromatic carbons, there are three oxygenated carbons, namely carbons at chemical shifts of 149.24; 114.79; 169.50 ppm.
- The carbon signal at the shift 213.22 ppm indicates the presence of carbonyl carbon C=O at C-4
- The signal at a shift of 73.95 ppm indicates oxygenated cyclic aliphatic carbon.

- The carbon signal at the chemical shift of 64.50 ppm indicates an unprotected cyclic methylene (CH₂) carbon.

Table 1. Tabulation of ¹H NMR and ¹³C-NMR spectroscopy data for Isolate F_{8.3} Japanese papaya

Position	δ NMR	
	δ H	δ C
2	3.58 <i>dd</i> (4.9; 16.8)	73.95
3a	3.51 <i>dd</i> (11.2; 4.9)	64.50
3b	3.65 <i>dd</i> (11.2;16.8)	-
4	-	213.22
5	-	149.24
6	7.71 <i>dd</i> (3.5; 5.6)	140.26
7	5.81 <i>m</i>	129.97
8	7.61 <i>dd</i> (3.5; 5.6)	73.66
9	-	133.67
10	-	125.32
1'	-	132.54
2'	7.45 <i>dd</i> (2.1; 4.2)	120.14
3'	-	114.79
4'	-	169.50
5'	7.44 <i>dd</i> (2.1; 8.4)	140.6
6'	7.21 <i>dd</i> (2.1; 8.4)	120.14

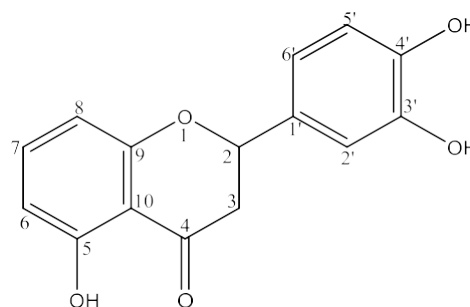


Figure 1. Proposed Structure; 3',4',5-trihydroxyflavanone

Based on the tabulated data (Table 1), isolate F_{8.3} from the ethyl acetate fraction of Japanese papaya leaves was compared with the chemical shift reported in the literature by Funari *et al.* (2011)[10], where the isolate shows the same flavanone nucleus as the literature, but the isolate does not have a monosaccharide unit at the C-7 position and an oxygenated carbon at the C-6 position, because at the C-6 and C-7 positions in

the isolate there are still proton signals appearing at chemical shifts of 6.82 and 7.71 ppm..

Conclusion

The results of the identification of isolate F_(8.3) from the ethyl acetate fraction of Japanese papaya leaves using NMR spectroscopy ¹H and ¹³C show a flavanone derivative compound with a proposed structure of 3',4',5-trihydroxyflavanone.

Reference:

1. Pongoh, E. J.; Rumampuk, R. J.; Howan, D. Skrining Fitokimia dan Potensi Antilitiasis dari Ekstrak Etanol Daun Nusa Indah Putih (*Mussaenda pubescens*). *Fullerene Journal of Chemistry*, 2019, 4(2), 76-81, DOI: [10.37033/fjc.v4i2.98](https://doi.org/10.37033/fjc.v4i2.98).
2. Saifudin, A.; Rahayu, V.; Teruna, H. Y., *Standarisasi Bahan Obat Alam*. Graha Ilmu: Yogyakarta, 2011; Vol. 4, p 69-84.
3. Werdiningsih, W.; Legowo, A. W. Identifikasi Senyawa Flavonoid Terhadap Rendemen Ekstrak Daun Pepaya Jepang (*Cnidoscopus aconitifolius* Mill) Dengan Metode Ekstraksi Sokhletasi. *Jurnal Pharma Bhakta*, 2023, 3(2), 57-65.
4. Adeniran, O. I.; Olajide, O. O; Igwemmar, N. C; Orishadipe, A. T. Phytochemical constituents, antimicrobial and antioxidant potentials of tree spinach (*Cnidoscopus aconitifolius* (Miller) I.M. Johnston), *Journal of Medicinal Plants Research*, 2013, 7(19), DOI: [10.5897/JMPR12.899](https://doi.org/10.5897/JMPR12.899).
5. Awoyinka, O. A., Balogun, I. O., & Ogunnowo, A. A. (2007). Phytochemical screening and in vitro bioactivity of *Cnidoscopus aconitifolius* (Euphorbiaceae). *Journal of Medicinal Plants Research*, 1(3), 63-65.
6. Jiménez-Aguilar, D. M.; Grusak, M. A. Evaluation of minerals, phytochemical compounds and antioxidant activity of Mexican, Central American, and African green leafy vegetables. *Plant foods for human nutrition*, 2015, 70(4), 357-364.
7. Cyril-Olutayo, M. C.; Adeyemo, T. A.; Oriola, A. O; Agbedahunsi, J. M. Bioactivity-directed isolation of antisickling Compounds from *Cnidoscopus aconitifolius* (Mill.) I.M Johnst leaf extract. *Journal of Pharmacognosy and Pharmacology Research*, 2020, 8(6), 5
8. Prayitno, S.; Andriana, A. N. I. Uji Efek Analgetik Fraksinasi Ekstrak Etanol Batang Brotowali (*Tinospora crista* L.) Terhadap Mencit (*Mus musculus*). *Fito Medicine: Journal Pharmacy and Sciences*, 2019, 11(2),1-9.
9. Funari, C. S; Passalacqua, T. G; Rinaldo, D; Napolitano, A; Festa, M; Capasso, A; Silva, D. H. S. (2011). Interconverting flavanone glucosides and other phenolic compounds in *Lippia salviaefolia* Cham. ethanol extracts. *Phytochemistry*, 1011, 72(16), 2052-2061.
11. Aloanis, A. A.; Fahriana, F.; Haryadi, H., *Skrining fitokimia dan uji toksisitas ekstrak daun balik angin (Mallotus Sp) terhadap larva Artemia salina Leach dengan metode brine shrimp lethality test (BSLT)*. *Fullerene Journal of Chem*
12. Moumou, F.; Thomas, A. A.; Kainde, R. P.; Nurmawan, W., *Pemanfaatan tumbuhan obat oleh masyarakat Desa Toliwang Kecamatan Kao Barat Kabupaten Halmahera Utara*. *COCOS* 2015,6, (12)
13. Peixoto Sobrinho TJS, Castro VTNA , Saraiva AM, Almeida DM, Tavares EA, Pisciotano MNC, Amorim ELC (2012). Phytochemical screening and antibacterial activity of four *Cnidoscopus* species (Euphorbiaceae) against standard strains and clinical isolates. *J. Med. Plants Res.* 6(21):3742-3748
14. Atuahene CC, Poku-Prempeh B, Twun G (1999). The nutritive values of chaya leaf meal (*Cnidoscopus aconitifolius*) Studies with broilers chickens. *Anim. Feed Sci. Technol.* 77: 163-172.
15. Chikezie UN, Chijioke AN, Adjeroh LA, Ogbulie TE, Udensi JU, Oyirioha KC (2016) *An evaluation of the phytochemical and nutritional compositions of fresh leaves of Cnidoscopus aconitifolius [Miller] I.M. Johnston*. *Int J Res Stud Biosci* 4(6): 21-28



2026 by the authors. Licensee Fullerene Journal of Chem. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) (<http://creativecommons.org/licenses/by/4.0/>)