
PHYTOCHEMICAL STUDY OF BIDARA UPAS (*Merremia mammosa* (Lour.) Hallier f.) LEAF

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ABSTRACT

Background: Respiratory diseases were reported in the displaced population after natural disasters. Bidara upas (*Merremia mammosa* (Lour.) Hallierf.) napiform root tuber has been used to treat diseases related to respiratory system, but few study about its leaf chemical compounds.

Objective: The research aimed to study one of the compounds from bidara upas leaf that might be functional in treating respiratory diseases.

Methods: This research began with phytochemical screening, extraction by graded maceration method using n-hexane, ethyl acetate, and 70% ethanol as solvent, and continued by stage of isolation compound and chemical characterization of isolates collected. TLC monitoring on the three extracts was done using silica gel GF₂₅₄ as a stationary phase and mobile phase chloroform: ethyl acetate (4:1). Isolation was done by preparative-TLC using silica gel 60F₂₅₄ as a stationary phase and mobile phase chloroform: ethyl acetate (4:1) and 10% H₂SO₄ in methanol as the apparition spot reagent. Isolates were characterized by using the apparition spot of 10% sulfuric acid in methanol, 5% AlCl₃ in methanol, 1% FeCl₃ in water, and 0.2% DPPH in methanol, UV-visible spectrophotometer and Fourier Transformed Infrared Spectrophotometry (FTIR).

Outcome measured : Characteristics of one of the compounds isolated from bidara upas leaf.

Results : The result of phytochemical screening showed that almost all of the three extracts contained flavonoids, quinones, phenolic compounds, triterpenoids and steroids. However, flavonoid was not detected in n-hexane extract. TLC monitoring on the three extracts showed the presence of well-separated compounds at R_f 0.73. The spectrum of UV-visible spectrophotometer showed that the isolates had a maximum absorbance at a wavelength of 442 nm. The result of analysis of the FTIR spectrophotometer showed some of functional groups on the wave number 3,371.3 cm⁻¹; 2,945.1 cm⁻¹; 2,833.2 cm⁻¹; 2,044.4 cm⁻¹; 1,656.7 cm⁻¹; 1,450.4 cm⁻¹; 1,421.4 cm⁻¹; 1,114.8 cm⁻¹ and 1,028.0 cm⁻¹.

Conclusion: In conclusion, based on the results of characterization, the isolate was assumed to be terpenoid compounds.

Keywords: Bidara upas leaf (*Merremia mammosa* (Lour.) Hallier f.), phytochemical study, terpenoid, spectrophotometer UV-Visible, FTIR.



INTRODUCTION

Bidara upas (*Merremia mammosa* (Lour.) Hallier f.) has empirically benefits for treating various diseases. Its napiform root tuber has been used to treat diseases related to respiratory system, which were reported in the displaced population after natural disasters (WHO, 2006: 7; Watson *et al.*, 2007: 3) such as coughs, difteria, shore throat, and pneumonia, and also treat other diseases such as fever, apendicitys, typhus, constipation, diabetes mellitus, snake bitten, and also cancer. Oxidase content of fresh exudate (latex) was hypothesized to have a role in cancer medication (Trubus, 2012:254-256) and as lactagogum (Sirait, *et al.*, 1993:124). The seeds was used for treating leprosy (Ogata, *et al.*, 1995:241).

Bidara upas was one of the rare species and there were few research about its phytochemicals or pharmacological activites. Earlier studies used its napiform root tuber (Ukhrowi, 2012:1). Bidara upas leaf was rarely used and so far there were no research publications concerning the phytochemical content of the leaf.

Based on the information, this research aimed to study and characterize one of the compounds from bidara upas (*Merremia mammosa* (Lour.) Hallier f.) leaf that might be functional in treating respiratory diseases or other diseases.

METHODS

This research began with material preparation, phytochemical screening and natural product standardization, extraction and monitoring, continued by stage of isolation compound and chemical characterization of isolates collected.

1. Material preparation

Fresh bidara upas leaves were collected from Kediri, East Java. The leaves were washed using flowing water, chopped, and air-dried for two days before continued using drying cabinet with temperature of 46°C. Dry leaves were grinded and preserved in tight-closed container.

2. Natural product standardization

The standardization procedure consisted of specific parameters and non-specific parameters determination. The specific parameters were identity (macroscopic and microscopic evaluation), organoleptic, water-extractable matter, and ethanol-extractable matter. Non-specific parameters were water content, total ash, acid-insoluble ash, specific gravity, and lost on drying.

3. Phytochemical screening

Phytochemical screening was conducted on the content of alkaloid, flavonoid, saponin, quinon, tannin, and steroid/terpenoid.

4. Extraction and Thin Layer Chromatography Monitoring

Extraction was done by graded maceration method using n-hexane, ethylacetate, and ethanol as solvent. Powdered leaves was macerated for 2x24 hours using n-hexane to extract non polar substances. Remaceration was done every 24 hours. Filtrates were collected and the residue was air-dried to evaporate the remaining solvent. Maceration using ethyl acetate was applied to the dried residue for 2x24 hours to collect semi-polar substances, continued with maceration with 70% ethanol for 3x24 hours to accumulate polar substances. The filtrates were evaporated and Thin Layer Chromatography (TLC) monitoring on the three extracts was done

using silica gel GF₂₅₄ as a stationary phase and mobile phase chloroform: ethyl acetate (4:1).

5. Isolation and purity test

Selected extract was fractionated using preparative TLC to isolate one of chemical component, using silica gel 60F₂₅₄ as a stationary phase and mobile phase chloroform: ethyl acetate (4:1) and H₂SO₄ 10% in methanol as the apparition spot reagent. The plates used were pre-activated in 105°C for one hour to remove water. 50 mg of selected extract was diluted in 1 ml of the ethyl acetate and then dropped on the plates to form stripe. The elution process was performed after the chamber has been saturated. Selected band formed was removed and then diluted in methanol to complete isolation. Purity test was conducted on the isolate using two methods: (1) single elution-TLC with three different eluent composition (non polar, semi-polar, and polar) and, (2) two dimensional TLC.

6. Chemical characterization

Isolates were characterized by using the apparition spot of 10% sulfuric acid in methanol, AlCl₃ 5% in methanol, 1% FeCl₃ in water, and DPPH 0,2% in methanol, UV-visible spectrophotometry and Fourier Transformed Infrared Spectrophotometry (FTIR).

RESULTS AND DISCUSSION

Natural product standardization

a. Botanical standardization

(i) Macroscopic evaluation

The macroscopic evaluation showed that the material used in this research had the same characteristic as described in the literature. The bidara upas leaves were green, nearly rotund ovate in shape, pubescent, entire leaf margin, cordate leaf basal, acute-acuminate leaf tip (Mansur, 2001:370-371), no characteristic odors, and white-hairy abaxial surface. The leaves were 8.5-14.2 cm (length) to 8.7-14.5 cm (wide).

(ii) Microscopic evaluation

- *Fresh leaves*: Microscopic evaluation of fresh leaves was conducted to determine adaxial-abaxial surface characteristics. The result showed anisocytic stomatal type on abaxial surface (Figure 1), needle-shaped calcium oxalate crystals and trichomes (Figure 2), also vessel bundle with stair-type and spiral-type lignified cells (Figure 3).
- *Powdered dry leaves*: Microscopic evaluation of powdered dry leaves was conducted to determine the characteristic fragments of bidara upas leaf. The evaluation results showed some typical fragment identified as needle-shaped calcium oxalate crystals and trichomes, same as observed in fresh leaves (Figure 4). According to Cronquist (1981: 897), parenchymatic cells of Convolvulaceae will show some shape-type of calcium oxalate crystals, which can be identified as single or clumped crystals. Convolvulaceae leaves will also show some typical stomatal types, which can be identified as paracytic, and rarely anisocytic or anomocytic.

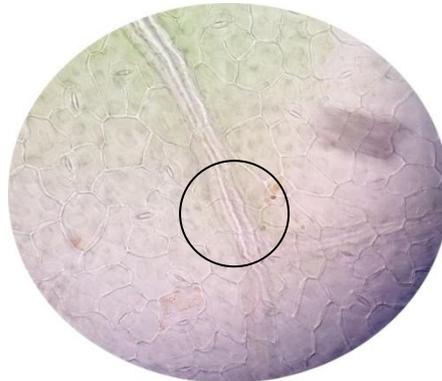


Figure 1. Anisocytic stomatal type on abaxial surface of fresh bidara upas leaf

b. Physical evaluation

Physical evaluation of bidara upas leaves was conducted on ash total value, acid insoluble ash value, water content, water soluble extractable matter, alcohol soluble extractable matter, and loss on drying (see Table 1).

Table 1. The result of bidara upas leaf physical evaluation

Physical evaluation parameters	Average values
Total ash	7.15 %
Acid insoluble ash	0.45 %
Total water content	6.48 %
Water-soluble extractable matter	18.96 %
Ethanol-soluble extractable matter	15.09 %
Lost on drying	7.77

(i) Ash value determination

Total ash value showed high inorganic matter content of the leaves (7.15%). High total ash value might be caused by internal (high mineral nutrition of the soil) or external factor (material preparation). Acid insoluble ash value showed that the material sample has lower value than MMI standard for acid insoluble ash maximum value. The value of acid\ insoluble ash should be lower than 2% (Depkes. RI, 1995: iii).

(ii) Water content determination

Water content standard for desired plant materials should not be more than 10 (%b/b) (Kemenkes. RI, 1994). Water content for bidara upas leaves in this research showed lower value than maximum standard value (6.48%). This value means that the bidara upas leaves in this research was validated due to MMI standard.

(iii) Extractable matter solubility determination

Extractable matter solubility in water had a higher value than extractable matter solubility in ethanol. In this case, extractable matter solubility values showed more polar soluble matter than semi-polar soluble matter.

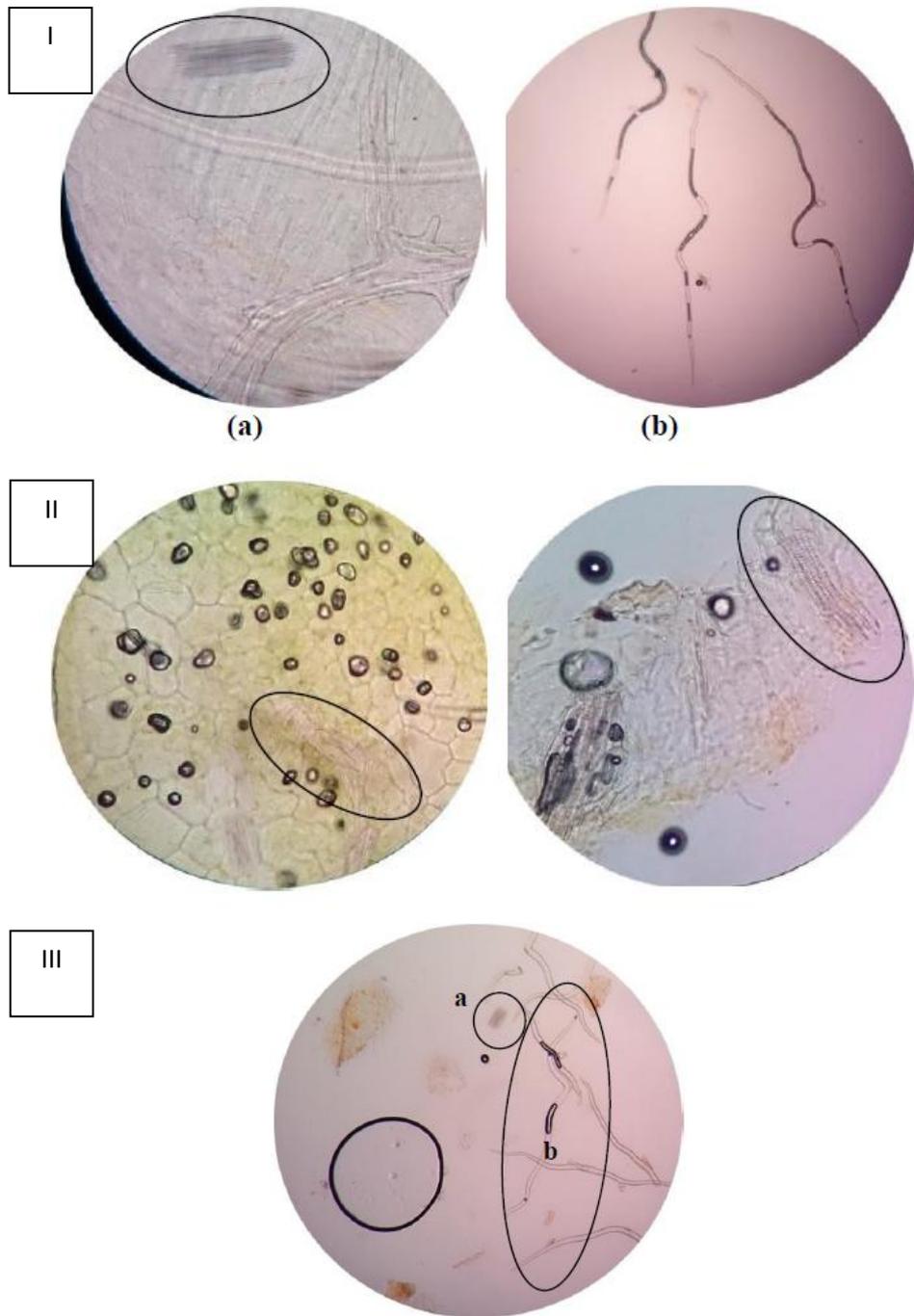


Figure 2. The result of microscopic evaluation on fresh bidara upas leaf, I. (a)needle-shaped calcium oxalate crystals, (b)trichome; II. (a)vessel bundle with stair-type cells, and (b)vessel bundle with spiral-type lignified cells; III. (a) needle-shaped calcium oxalate crystal, (b) trichomes



(iv) Loss on drying determination

Loss on drying determination aimed to give maximum limitation for the amount of compound loss under drying process (Depkes. RI, 2000:13). The lost compounds under drying are vaporable and volatile compound such as water and essential oil.

Phytochemical screening

Phytochemical screening result showed that almost all of the three extracts contained flavonoids, quinones, phenolic compounds, triterpenoids and steroids. However, flavonoid was not detected in n-hexane extract.

Table 2. The result of phytochemical screening of fresh, dried leaves, and extract of bidara upas

Phytochemical substance	Identification result				
	Fresh	Dried leaves	n-hexane extract	Ethyl acetate extract	70% ethanol extract
Alkaloid	-	-	-	-	-
Flavonoid	-	+	-	+	+
Saponin	-	-	-	-	-
Tannin	-	-	-	-	-
Quinone	+	+	+	+	+
Fenolic substance	+	+	+	+	+
Monoterpene & Sesquiterpene	-	-	-	-	-
Triterpene & Steroid	+	+	+	+	+

Details:
(+):identified
(-) : unidentified

As seen in Table 2, bidara upas fresh leaves contain detectable quinon, phenolic, triterpenoid, and steroid compounds. Dried leaves of bidara upas contain detectable flavonoid, quinon, phenolic, triterpenoid, and steroid. This results following Cronquist (1981: 897) that generally, Convolvulaceae plants has no saponins nor tannins.

In n-hexane extract, phytochemical screening showed detectable test for quinon, phenolic, triterpenoid, and steroid compounds, meanwhile in ethyl acetate and 70% ethanolic extracts contained detectable flavonoids, quinon, phenolic, triterpenoid, and steroid compounds.

Extract and TLC monitoring

Maceration was chosen because there was no information about the chemical content characteristics of bidara upas leaf. Graded maceration was done to extract the leaf chemical substance based on its polarity level, as mentioned by Jones & Kinghorn (2006: 329-330). The highest yield was showed by 70% ethanolic extract (32.6%), as displayed in Table 3. This might be resulted from the addition of some non-polar and semi-polar substances that still remained in the residue after n-hexane or ethyl acetate extraction, which was finally removed from the residue by 70% ethanol, and added to the polar substances. The highest specific gravity also showed by 70% ethanolic extract (Table 3).

Table 3. The yield and specific gravity values of bidara upas leaf extracts

Extract	Weight (gram)		Yield (%)	Specific gravity (mg/ml)
	Dry leaves	Extract		
n-hexane		9.59	1.26	1.06
Ethyl acetate	760	11.43	1.50	1.10
70% ethanol		247.81	32.6	1.14

The result of TLC monitoring on the three extract displayed in Figure 5. The chromatograms were examined under visible light and 254 & 365 nm Ultra Violet (UV) light. The appearance of the spots on the TLC plates were also made visible by using 10% H₂SO₄ dissolved in methanol. Ethyl acetate extract was chosen because it displayed the best separation with the presence of well-separated compounds at Retention Factor (RF) value of 0.73.

Isolation and purity test

Preparative TLC was done on ethyl acetate extract with silica gel 60 F₂₅₄ as stationary phase and chloroform : ethyl acetate (4:1) for the mobile phase. The observation was conducted under visible light. The results showed a yellow band at the same RF as displayed on the chromatograms of TLC monitoring (Figure 6), which was then removed to collect the isolate. The isolated compound was then submitted to purity test by single elution-TLC monitoring using three different mobile phase composition and two dimensional TLC monitoring using two different mobile phase composition.

On the first purity test, the isolate was monitored using non polar mobile composition (chloroform : ethyl acetate = 9 : 1) and gave rise to a spot at RF 0.38. Next, the semi polar mobile phase (chloroform : ethyl acetate = 4 : 1) was used and resulted a spot at RF 0.74. Last one, polar mobile phase (ethyl acetate : methanol = 9 : 0.5) was used and arose a spot at RF 0.88 (Figure 7).

Two dimensional TLC using silica gel GF₂₅₄ as the stationary phase was completed using two mobile phase composition: (1) the semi-polar mixture of chloroform : ethyl acetate (4:1), and (2) the polar mixture of ethyl acetate : methanol (8:1). The outcome of this test was a single spot which indicated that the isolate was pure (Figure 8).

Chemical characterization

The isolated compound was characterized using 10% H₂SO₄ dissolved in methanol, 1% FeCl₃ dissolved in water, and 0.2% DPPH dissolved in methanol (Figure 9). Using 10% H₂SO₄ and heated in 100-105°C, the isolate compound appeared yellow under the 365 nm UV light. According to Harborne (1996:135), this result indicated that the isolate was terpenoid. On the other hand, the isolate did not showed any changes when sprayed with



5% AlCl_3 in methanol or 1% FeCl_3 in water. This implied that the isolate was not flavonoid or fenolic compound. The result of spraying the isolate with 0.2% DPPH showed that the isolated compound had the antioxidant potency, along with the DPPH color change from purple to yellow.

From the analysis using UV-visible spectrophotometry (Figure 10), the isolated compound showed maximum wavelength at 442 nm (absorbance 0.673). Maximum absorbance detected over 250 nm indicated that the substance had conjugated double bond. Furthermore, the spectrum showed a similarity with carotenoid which the spectrum is very specific around 400-500 nm, with two main peak at 450 nm and two additional peak at both side of the main peak (Harborne, 1996:164-165).

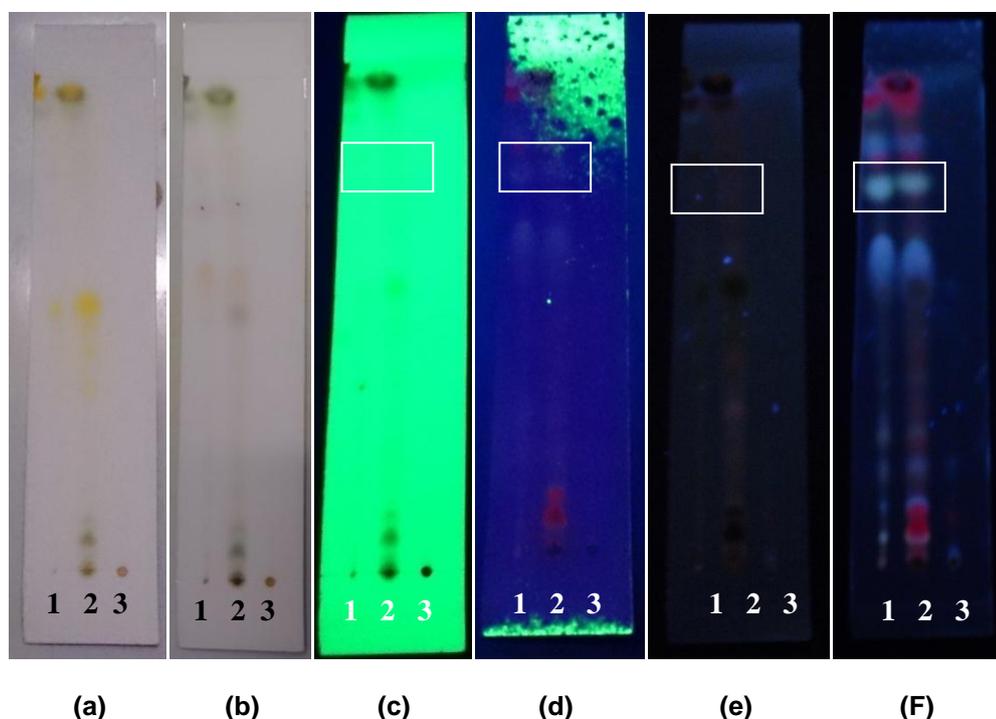


Figure 5. TLC monitoring chromatograms of (1) n-hexane extract, (2) ethyl acetate extract, (3) 70% ethanol extract. (a) before sprayed by 10% H_2SO_4 (under visible light), (b) after sprayed by 10% H_2SO_4 (under visible light), (c) before sprayed by 10% H_2SO_4 (under 254 nm UV light), (d) after sprayed by 10% H_2SO_4 (under 254 nm UV light), (e) before sprayed by 10% H_2SO_4 (under 365 nm UV light), (f) after sprayed by 10% H_2SO_4 (under 365 nm UV light). The white box indicated the spot at R_f 0.73.

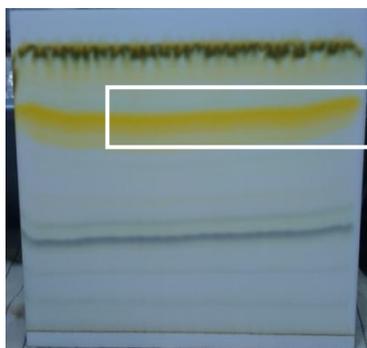


Figure 6. The chromatogram of preparative TLC (Stationary phase: silica gel 60 F₂₅₄; Mobile phase: chloroform : etyl acetate (4:1), under visible light). Yellow band pointed with the white box was removed to collect the isolate.

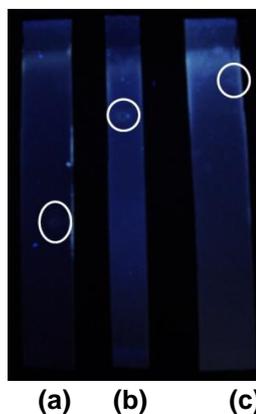


Figure 7. The result of single elution-TLC monitoring for purity test. Stationary phase: silica gel GF₂₅₄, observed under 365 nm UV light, mobile phase: (a) chloroform : ethyl acetate (9:1), (b) chloroform : ethyl acetate (4:1), (c) ethyl acetate : methanol (9:0.5). The white circles indicated the spots of the compound.

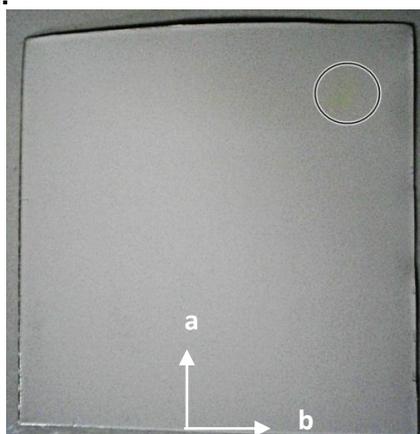


Figure 8. The result of two dimensional TLC using silica gel GF₂₅₄ (stationary phase) under visible light and mobile phase: (a) ethyl acetate : methanol (8:1), and (b) chloroform : ethyl acetate (4:1). The isolate was pointed with a circle.

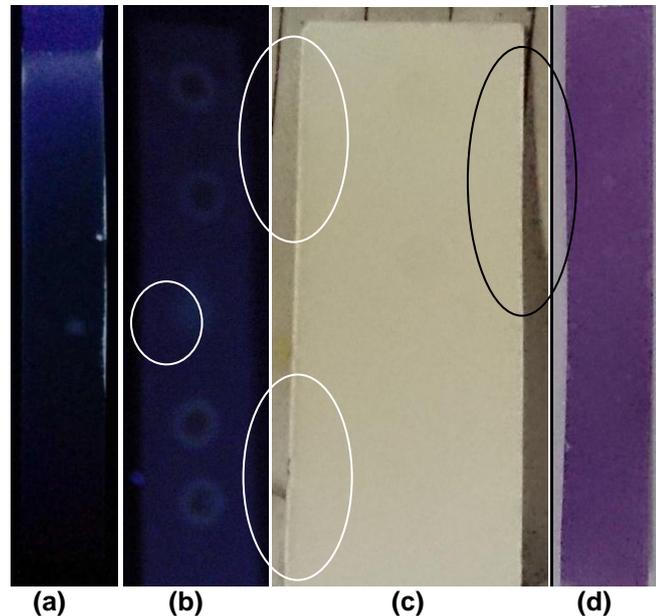


Figure 9. The result of isolate characterization using silica gel GF254 and spotting apparition: (a) 10% H_2SO_4 in methanol (mobile phase: chloroform : ethyl acetate = 9:1); (b) 5% $AlCl_3$ in methanol; (c) 1% $FeCl_3$ in water; (d) 0.2% DPPH in methanol. The circles indicated the isolated compound.

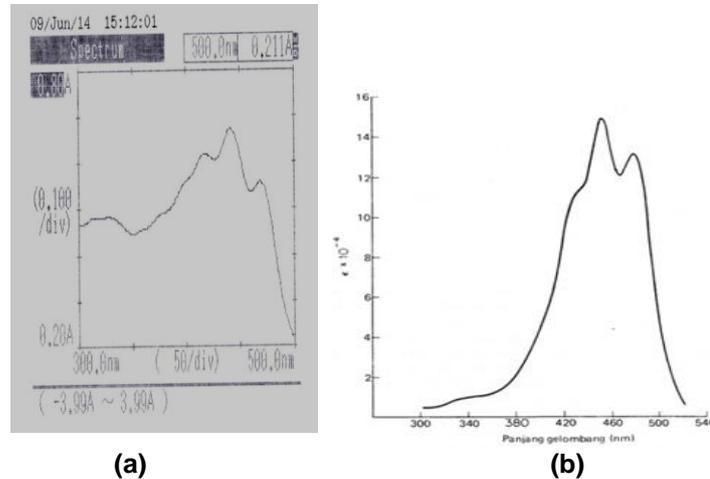


Figure 10. The result of UV-visible spectrophotometry analysis of the isolated compound: (a) UV-visible spectrum of isolate, and (b) visible spectrum of β -carotene (Harborne, 1996:165)

The result of isolated compound analysis using FTIR spectrophotometer showed some of functional groupson thewave numbers revealed (Table 4).

Based on this result combined to TLC-monitoring and UV-Visible spectrophotometry results, confirmed with explanations by Harborne (1996:135, 164-166), it was concluded that the isolated compound from Bidara Upas leaf was suspected to have the same characteristics as terpenoid.

Table 4. The result of functional groups interpretation of isolated compound using FTIR spectrophotometry

No.	Wave number (1/cm)	Transmittant (%)	Functional Groups (Fessenden & Fessenden, 1986: 314-326)	Wavelength Range (1/cm)
1	1028	34	C-O, C-C, C-N	1300-900
2	1114.8	64	C-O, C-C, C-N	1300-900
3	1421.4	55	C-H, C-C, O-H	1600-1450
4	1450.4	52	C-H, C-C, O-H	1600-1450
5	1656.7	70	C=O, C=N, C=C, N=O	1700-1600
6	2044.4	78	C≡C, C≡N	2300-2000
7	2833.2	32	C-H alifatik	3000-2800
8	2945.1	31	C-H	3000-2800
9	3371.3	24	O-H	3700-3000

CONCLUSION

In conclusion, based on the results of characterization, the isolate was assumed to be terpenoid compounds.

DISCLOSURE

There is no conflict of interest between the writers.

ACKNOWLEDGEMENT

The writers would like to acknowledge Universitas Islam Bandung for supporting this research and publication.

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