

# Pharmacognostic and Cytotoxicity Evaluation of Indonesia Native Plant of *Piper acre* Blume Leaves (*Piperaceae*)

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## History

- Submission Date: 16-12-16;
- Review completed: 05-01-17;
- Accepted Date: 27-02-17.

DOI : 10.5530/pj.2017.3.68

## Article Available online

<http://www.phcogj.com/v9/i3>

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## ABSTRACT

*Piper acre* Blume (Piperaceae) is an Indonesia native plant that is used as a traditional medicine and commonly known as 'Sirih Hitam' or 'Sirih Hutan'. The scientific data from the plant is still very limited. The aims of the present study was to know a pharmacognostic, and cytotoxicity of *P. acre* Blume leaf. The macroscopic characters showed that the leaves have a characteristic odor, heart-shaped, margin was finely flat, venation was curved, the upper surface was dark green and slippery while the lower surface was pale green and soft. The microscopic characters revealed upper and lower epidermis cells, glandular trichomes, concentric vascular bundle, paracytic stomata. The fluorescence characteristics showed a variety of chemical constituents present in the plant materials. The preliminary phytochemical investigation of various extracts showed the presence of alkaloid, flavonoid, saponin, terpenoid, tannin, carotenoid, and steroid. The physicochemical properties (yield, color, consistency) of the n-hexane, ethyl acetate, and ethanol extract were 31.57% w/w (green concentrated solid), 13.59% w/w (green concentrated solid), and 16.35% w/w (brownish green liquid). Based on the cytotoxicity using brine shrimp lethality assay of the n-hexane extract (2.46 µg/ml), ethyl acetate extract (0.925 µg/ml), and ethanol extract (5.741 µg/ml) showed that the extract had the cytotoxic activity.

**Key words:** *Piper acre* Blume, pharmacognostic, preliminary phytochemical, physicochemical, cytotoxicity.

## INTRODUCTION

The genus of *Piper* has more than 1,000 species, which is one of the most diverse genera of the shrubs, herbs, and Liana commonly found in the lowland wet forest. The diversity of the genus occurs in Tropical America (700 species) and South Asia (300 species).<sup>1,2</sup>

Empirically, the species from the genus of *Piper* had been widely used in traditional medicine such as *P. betle*, *P. nigrum*, *P. piscatorum*, *P. dariensis*, *P. longum*, *P. auritum*, *P. sarmentosum*, *P. amalago* and many other species. Researchers had reported some activities of these plants, such as anesthetic, anti-inflammatory, analgesic, antibacterial, antidepressant, antioxidant, and anticancer.<sup>3-6</sup> Phytochemical investigations from many species of *Piper* had isolated biologically active compounds including lignans, alkaloids, flavones, aristolactams, unsaturated amides, monoterpenes, long and short chain esters, sesquiterpenes, arylpropanoids, aldehydes, ketones, propenylphenols, and chalcones.<sup>7</sup> Until now, there are still many other species that has not examined, one of which is *Piper acre* Blume.

*P. acre* Blume is an Indonesian native plant that used as a traditional medicine and commonly known as 'Sirih Hitam' or 'Sirih Hutan' with spread growth in Java Island and East Kalimantan. Empirically, this plant used as a traditional medicine for various diseases by Indonesian (a particularly local community in East Kalimantan), such as itching due to infection, cysts, prostate, pain (abdominal pain), and jaundice.

However, the scientific data is still very limited. This plant had been conducted toxicity tests using animal models. The test results showed that the water extract of *P. acre* Blume proved not caused liver necrosis based on changes levels of SGPT and SGOT<sup>8</sup> and also had reported antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method.<sup>9</sup> Therefore, it becomes more interesting to explore pharmacognostic profile of this plant. The pharmacognostic study related to authentication, standardization, and natural products study.

## MATERIALS AND METHOD

### Plant Collection and Authentication

Leaves of *Piper acre* Blume (*P. acre* Blume) were collected from Samarinda, East Kalimantan, Indonesia. Moreover, this plant was identified at Laboratory of Biosystematics, Indonesian Institute of Science (LIPI) – Cibinong, West Java, Indonesia. Moreover, the voucher specimen was deposited at Laboratory of Research & Development of FARMAKA TROPIS, Mulawarman University.

### Macroscopic Evaluation

Macroscopic evaluation of *P. acre* Blume leaves based on the method in some literature.<sup>10-12</sup> Fresh, dried, and powder of leaves sample was observed by organoleptic (shape, size, color, and odor).

**Cite this Article:** Ahmad I, Ambarwati NSS, Arifuddin M, Rijai L, Mun'im A. Pharmacognostic and Cytotoxicity Evaluation of Indonesia Native Plant of *Piper acre* Blume Leaves (*Piperaceae*). Pharmacogn J. 2017;9(3):400-4.

## Microscopic Evaluation of Fresh Leaf and Dried Powder

Transversal and longitudinal sections of the fresh leaves prepared using the standard procedures.<sup>10,13-15</sup> Both sections were observed under microscope Nikon Eclipse E200 (10X and 40X), photomicrographs of specimens were taken using Nikon Coolpix 4500 camera (4,0 megapixel). Dried leaves powder was carried out using the chloral hydrate and phloroglucinol-HCl reagent, and also was observed under microscope Nikon Eclipse E200 (10X and 40X), photomicrographs of specimens were taken using Nikon Coolpix 4500 camera (4,0 megapixel).

## Fluorescence Evaluation

Fluorescence study of the dried leaves powder performed using standard procedure.<sup>11,12,16</sup> A small quantity of the sample treated using fluorescence reagents (such as 1 N NaOH, 1 N HCl, 50% H<sub>2</sub>SO<sub>4</sub>, FeCl<sub>3</sub>, Iodine solution, Acetic Acid Glacial, 0,1 N NH<sub>4</sub>OH, 1% CH<sub>3</sub>COOH). Moreover, analyzed under visible light, short ultra-violet light (wavelength 254 nm), and long ultra-violet light (wavelength 366 nm).

## Preliminary Phytochemical Evaluation

The dried leaves powder were macerated successively with different solvent (n-hexane, ethyl acetate, and ethanol).<sup>17,18</sup> The sample was macerated successively with n-hexane, ethyl acetate, and n-butanol for 24 hours. Extracts solution was evaporated to obtain dried extracts and analyzed using chemical reagent for preliminary phytochemical investigation of various groups of phytoconstituents such as alkaloids, flavonoids, saponins, terpenoids, tannins, carotenoids, and steroids by using the standard procedure.

## Physicochemical Evaluation

This study was just using physicochemical parameters like percentage yield (% w/w), color and consistency of the extracts.<sup>12</sup>

## Cytotoxicity Evaluation

The cytotoxicity evaluation was performed using brine shrimp lethality test (BSLT) assay according to the method describe in the literature with modification.<sup>19-21</sup> Each of the extracts was tested at 40, 20, 10, 5, 2.5, 1.25, and 0.75 µg of extract per ml (in seawater). The concentration was achieved by transferring the appropriate volume from the stock solution; it performed by inserting ten brine shrimp aged 48 hr (mainly nauplii in instar III/IV) into the vessels containing the test solution extracts and incubated at 28–30°C with strong aeration, under a continuous light regime. Moreover, the number of survivors counted and calculated, and then analyzed using a statistical method (regression linearity) to determine LC<sub>50</sub>.

## RESULTS

### Authentication and macroscopic evaluation

The classification of this plant through the process of identification at the Indonesian Institute of Sciences (LIPI) - Cibinong, West Java, Indonesia, and according to Cronquist<sup>22</sup> as follows:

Family : Piperaceae

Genus : Piper

Species : *Piper acre* Blume.

Fresh leaves of *P. acre* Blume was dark green. The leaves is simple, have a characteristic odor and its heart-shaped with average leaf size is 4.5 – 8 cm length and 3 – 6 cm breadth. Apex (*Apex folii*) tends to taper (acuminate). Base (basis folii) is notched (*emerginatus*), the margin is finely flat, venation was curved like lines curved, the upper surface is dark green and smooth while the lower surface is pale green and soft, and also dried

leaves is blackish green. Authentication and macroscopic characteristics of the leaves shown in Figure 1A.

### Microscopic evaluation of the fresh and dried powder

Photomicrographs of the fresh of *P. acre* Blume leaf showed in Figure 1B. The transverse section revealed that the cells of lower epidermal cells were comparatively larger than the upper one, the reason is that the lower epidermis cells have many glandular epidermal hair (glandular trichomes) with contained oil glands. A single layer of cuticle covered the epidermis cells. About 4-6 layers of cortex (spongy mesophyll cells) surrounded the concentric vascular bundle wick the cortex consists of spherical shaped parenchymatous cells. The longitudinal section of the leaves presenting stomata on adaxial only (Hypostomatic leaf) with the paracytic types.

The most common features from the leaves' powder were stomata with paracytic types, spiral vessels element, oil glands with reddish yellow colors, and glandular trichomes (Figure 3C). This characteristic was observed and reported for the first time report.

### Fluorescences evaluation

The fluorescences characteristics of the dried leaves powder analyzed under visible light, short ultra-violet, long ultra-violet after treatment with organic and inorganic reagents (summarized in Table 1). Fluorescence is a characteristic shown by a variety of chemical constituents present in the plant materials. The chemical constituents that had been treated using different reagents would fluoresce at a specific wavelength especially in visible light, short ultra-violet light (254 nm wavelength), and long ultra-violet light (366 nm wavelength).

### Physicochemical evaluation

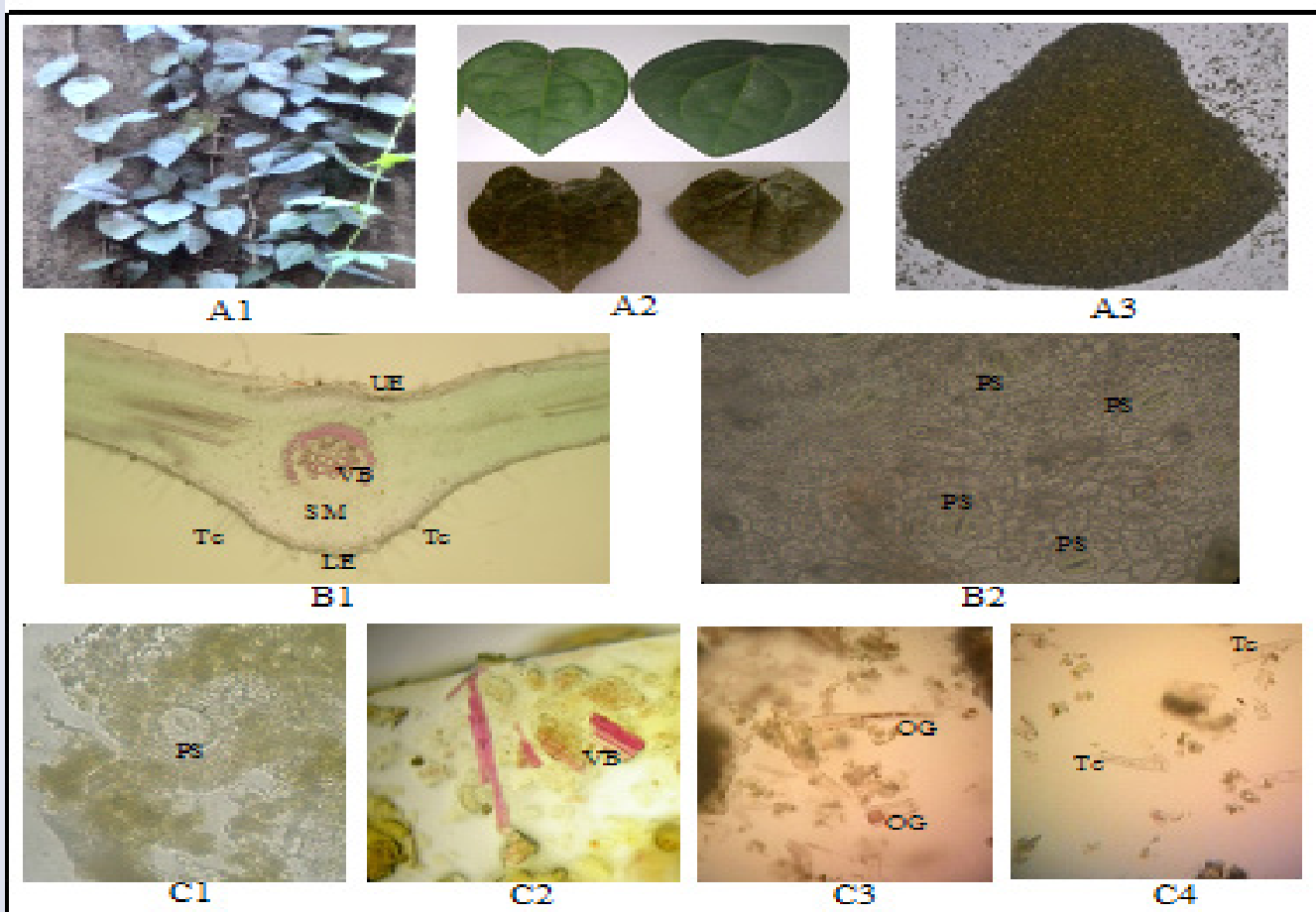
The physicochemical evaluation using percentage yield (% w/w), color and consistency parameters of the extracts. The percentage yield successive highs on n-hexane, ethanol, and ethyl acetate extracts, wherein the n-hexane extract was nonpolar and contains much chlorophyll, the ethyl acetate extract possibilities are only a few chlorophyll. However, the extract can attract the compound types that vary especially the compound groups which have moderate polarity. While on the ethanol extract that can attract polar compounds. The percentage yield (% w/w) with color and consistency, mentioned in Table 2.

### Preliminary phytochemical evaluation

The preliminary phytochemical investigation of the extracts showed the presence of chemical constituents from each extract based on different polarity levels of solvents (n-hexane, ethyl acetate, and ethanol). N-hexane extract showed the presence of flavonoids, terpenoids, carotenoids, and steroids. Ethyl acetate extract showed the presence of alkaloids, flavonoids, saponins, terpenoid, carotenoids, and steroids, and Ethanol extract showed the presence alkaloid, saponins, tannins, carotenoids, and steroids, mentioned in Table 3.

### Cytotoxicity valuation

The cytotoxicity evaluation using Brine Shrimp Lethality Test (BSLT), the LC<sub>50</sub> was obtained with n-hexane, ethyl acetate, and ethanolic extracts (showed in Table 4). The results achieved in the present study indicate the cytotoxic activities found in each extract, and the most powerful was the ethyl acetate extract (LC<sub>50</sub> = 0.925 µg/ml). Although, all of them were categorized as cytotoxic agent (each LC<sub>50</sub> values of less than 30 µg/ml was considered cytotoxic).<sup>8,21</sup>



**Figure 1:** Macroscopic and Microscopic characteristics of *Piper acre* BLUME.

Where; (A1) *Piper acre* Blume in natural habitat; (A2) a part showing the fresh and dried leaf; (A3) dried leaf powder; (B1) Transversal and (B2) longitudinal sections of the fresh leaf, (C) dried leaf powder; (UE) upper epidermis; (VB) vascular bundle; (Tc) Trichome; (SM) spongy mesophyll cell; (LE) lower epidermis; (PS) paracytic stomata; and (OG) oil glands.

**Table 1: Fluorescence Characteristics of Dried Leaves Powder of *Piper Acre* Blume**

Powder + Reagent	Colour Observed in Ordinary light	Colour Observed Under UV Short (254 nm)	Colour Observed Under UV long (366 nm)
Powder as such	Yellowish green	Yellowish green	Yellowish green
Powder + 1 N NaOH in water	Yellowish green	Brownish black	Greenish black
Powder + 1 N HCl	Brownish green	Brownish green	Greenish black
Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Brownish black	Reddish black	Greenish black
Powder + FeCl <sub>3</sub>	Greenish black	Greenish black	Greenish black
Powder + Iodine water	Reddish black	Reddish black	Greenish black
Powder + Acetic Acid Glacial	Yellowish green	Greenish black	Greenish black
Powder + 0.1 N NH <sub>4</sub> OH	Brownish black	Brownish black	Greenish black
Powder + 1% CH <sub>3</sub> COOH	Brownish black	Brownish black	Greenish black

**Table 2: Nature and Percentage Yield of the Different Extracts of Dried Powdered Leaves of *Piper acre* Blume**

Extracts	Colour & consistency of extract	Percentage Yield (% w/w)
n-Hexane	Green concentrated solid	31.57
Ethyl acetate	Green concentrated solid	13.59
Ethanol	Brownish green liquid	16.35

**Table 3: Preliminary Phytochemical Investigation Performed in the Various Extracts Dried Leaves Powder of *Piper Acre* Blume**

Phytochemical Test	N-Hexane	Ethyl acetate	Ethanol
Alkaloids	-	+	+
Flavanoids	+	+	-
Saponins	-	+	+
Terpenoids	+	+	-
Tannins	-	-	+
Carotenoids	+	+	+
Steroids	+	+	+

Note: + (Present), - (Absent)

**Table 4: Cytotoxic Activity Evaluation Using Brine Shrimp Lethality Test (BSLT)**

Extracts	Lethal Concentration (50%) using BSLT method ( $\mu\text{g/ml}$ )
n-Hexane	2.460
Ethyl acetate	0.925
Ethanol	5.741

## DISCUSSION

Based on the history of pharmacognosy sciences, plants have used in traditional medicine systems throughout the world. Research on medicinal plants is rapidly increasing on a global level. Further study with emphasis on efficacy, safety, and quality of natural products in health care system. The pharmacognostic analysis has an important role in the authentication and standardization of a medicinal plant because it is a necessary step for herbal drugs and their formulation in the traditional system of medicine.<sup>23,24</sup> The pharmacognostic character of crude extract is an initial information in standardization to maintain the purity, quality, and efficacy of crude drugs.<sup>18</sup> Authentication and macroscopic evaluation are helpful to ensure the authenticity of crude drugs to evaluate the external appearance including the size, shape, color, taste, texture, smell, and so on. Microscopic evaluation of the fresh leaf sample is necessary for photomicrographs identification of microscopic characteristics (particularly types of stomata, vessels element, oil glands, and glandular trichomes) which can distinguish with other species using optical microscopy. While, identifying the dried powder is useful for microscopical authentication based on the characteristics of the fresh sample. Fluorescence evaluation is an important parameter that most frequently used and assessed qualitatively for pharmacognostic evaluation of crude drugs. The physicochemical evaluation using percentage yield (% w/w), color and consistency parameters is useful in the identification and authentication of crude extract. Preliminary phytochemical evaluation will express the chemical contents of each extract. Also, the activity evaluation will reveal the potential of active chemical constituents in each extract.

In the present work is preliminary data (specifically pharmacognostic and cytotoxic activity data) for further studies. Moreover, this data was first time reported from this plant. In addition to assist in establishing parameters for identification of raw materials and preparation of plant monograph but also will help in the development of future studies.

## CONCLUSION

The results of this study are preliminary data for future studies and serves as a pharmacognostic parameter. This parameter was first time reported from this plant. In addition to assist in establishing parameters for identification of raw materials and preparation of plant monograph but also will help in the development of future studies.

## ACKNOWLEDGEMENT

The author is thankful to Head Pharmaceutical Research & Development Laboratory of FARMAKA TROPIS, Mulawarman University, and Head Laboratory of Pharmacognosy and Phytochemistry of Pharmacy Faculty, Universitas Indonesia for providing facilities, and also many thanks to Dean Pharmacy Faculty of Mulawarman University for financially supporting the study.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest

## ABBREVIATIONS USED

SGPT: Serum Glutamic Pyruvate Transaminase; SGOT: Serum Glutamic Oxaloacetic Transaminase; DPPH: 1,1-diphenyl-2-picrylhydrazyl; LIPI: Lembaga Ilmu Pengetahuan Indonesia; BSLT: Brine Shrimp Lethality Test;  $LC_{50}$ : Lethal Concentration-50; UE: upper epidermis; VB: vascular bundle; Tc: Trichome; SM: Spongy Mesophyll cell; LE: Lower Epidermis; PS: Paracitic Stomata; and OG: Oil Glands.

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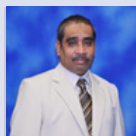
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**Cite this Article:** Ahmad I, Ambarwati NSS, Arifuddin M, Rijai L, Mun'im A. Pharmacognostic and Cytotoxicity Evaluation of Indonesia Native Plant of *Piper acre* Blume Leaves (*Piperaceae*). *Pharmacogn J.* 2017;9(3):400-4.