Antioxidant and Cytotoxic Activity of *Phyllanthus acidus* Fruit Extracts

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Abstract. *Phyllanthus acidus* is an Indonesian plant belonging to the Euphorbiaccae family. Extraction of *P. acidus* was performed using the maceration method. Four-solvent extraction process by ethanol, 70% ethanol, 30% ethanol, and water was used. The antioxidant activity from this extract was determined using 2,2-diphenyl-1-pikrilhidrazil (DPPH) and cytotoxicity (biological potency from this extract) using Brine Shrimp Lethality Test (BSLT) method. We found that the extraction yields of samples ranging from 1.13% to 20.25%. The ethanol extract showed the highest yield, while the lowest yield was reported in the water extract of *P. acidus* fruit. Among the samples, water extract of *P. acidus* exhibited high antioxidant activity with IC_{50} 26.06 µg/mL. LC_{50} values for BSLT ranging from 473.26 to 908.98 µg/ mL, with the water extract having the lowest value and therefore the most potent, and the ethanol extract having the highest value

1. Introduction

Oxygen is a paradox in aerobic organism [1]. An organism needs oxygen for energy production by respiration. However, oxygen also undergoes reaction into superoxide anion radical, hydrogen peroxide, and hydroxyl radicals. Those molecules are free radicals and cause oxidative stress to cells. Oxidative stress causes biomolecular damage by attack of reactive oxygen species (ROS). In human, oxidative stress related to various degenerative process and diseases.

Phyllantus acidus (L.) Skeel species belongs to the Plantae kingdom, Spermatophyta division , Angiospermae sub division, Dicotyledonae class , Euphorbiales order ,Euphorbiaceae family , and *Phyllanthus* genus . It can be found in the Caribbean and Pacific Ocean part of Southeast Asia. Their local names are grosella (Puerto Rico), jimbilin (Jamaica), karamay (Philippines), and ciremai (Indonesia and Malaysia) [2]. *P. acidus* tree can grow up to 9 m in height. The tree has tough main branches and long branchlets. Leaves are densely arranged in the branch. The leaves size is 2 to 7.5 cm long with a light green color. The flowers are male, female, or hermaphrodite. Flower size is 5 to 12.5 cm long with pink color. Fruits are 2 cm in diameter and densely clustered. Fruit is edible and the color is white to yellow. Fruits contain high moisture and taste sweet and juicy [3].

In Indonesia, every part of the tree is believed to have different medicinal properties. The fruits are used as astringent, laxative, and to heal ulcer. The leaves are used to treat cancer, cough, asthma, scurvy, and reduce body weight. The bark is used for asthma and rash. Local people consume the fruit directly or as an ingredient in pickle. For medication, 25 g of plant material is mixed with 200 mL of water [3].

Cytotoxic, antibacterial, and antioxidant activity of *P. acidus* fruit petroleum ether extract was reported by Habib *et al*. [4]. *P. acidus* leaves activity as antiinflamatory, antinociceptive, and antioxidant have been reported by Chakraboty *et al* [5]. and antihyperlipidemic activity of *P. acidus*

fruit acetone and methanol extract was reported by Andrianto *et al*. [2]. However, antioxidant and cytotoxic activity of *P. acidus* fruit extracts in ethanol and aqueous solvents have not yet been reported before. This research was carried out to evaluate antioxidant and cytotoxic activity of *P. acidus* fruit extracts.

2. Experimental

2.1 Plant materials

The plants were collected in Bekasi, Bogor, and Sumedang region of West Java Province, Indonesia. Plants were identified by Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

2.2 Materials

2-(N-morpholino)ethanesulfonic acid (MES) was from Dojindo Lab. (Tokyo, Japan). Trolox, 1,1-diphenyl-2-picrylhydrazyl (DPPH). Other chemicals used were analytical grade.

2.3 Extraction

Fresh fruits were washed and soaked in 5% NaCl to prevent browning. It was then blended into a fine juice and freeze-dried immediately. Dried samples were ground into powder and extracted using ethanol, 70% ethanol, 30% ethanol, or water (5 times volumes) at room temperature for 3 days. The macerates were filtered and the process was repeated one more time. Mixture was filtered using Whatman filter paper (No. 2), the solution was collected to become extract. Both acetone and methanol extract were concentrated under reduced pressure on rotary evaporator. The concentrated extracts were then used for the experiments.

2.4 DPPH radical scavenging activity

The antioxidant activities of the extracts were assayed for the effect of scavenging DPPH free radicals based on the method described by Blois [6]. Sample was added into a mixture (0.9 mL) of 0.4 mM DPPH solution, 20% methanol aqueous solution, and 0.2 M MES buffer solution to make final concentration 0, 30, 60, 90, 120, and 150 ⌈g/mL. Absorbance was measured at 520 nm. Trolox was used as positive control. The inhibitory activity was calculated from the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{1-A}{A_0}\right) \times 100\% \quad (1)$$

where A_0 is the absorbance of the mixture without sample and A is the absorbance of the mixture with a sample.

2.5 Brine shrimp lethality test (*Nurcholis* et al.) [7]

Ten *Artemia salina* Leach were incubate in 100 ⌈L sea water in a well plate. It was added with 100 ⌈L sample in 0, 10, 100, 200, 500, and 1000 ⌈g/mL concentration, respectively. Treatments were done with 3 replications. Mixture was gently shaken and incubates for 24 hours in room temperature. Dead *A. salina* from each well was counted and LC_{50} values were calculated based on probit analysis.

3. Result and Discussion

3.1 Extraction

Fresh *Phyllanthus acidus* fruit samples were used in the research. Water content in our samples were 85.55±3.00%. High water content supports the growth of the fruits while it attached on the trees. However, it will cause the fresh fruit easily broken after harvesting process.

Extraction processes were carried out by using ethanol, 70% ethanol, 30% ethanol, and water (figure 1). We obtained the yield of each extracts are 20.25%, 3.72%, 3.28%, and 1.13%, respectively.

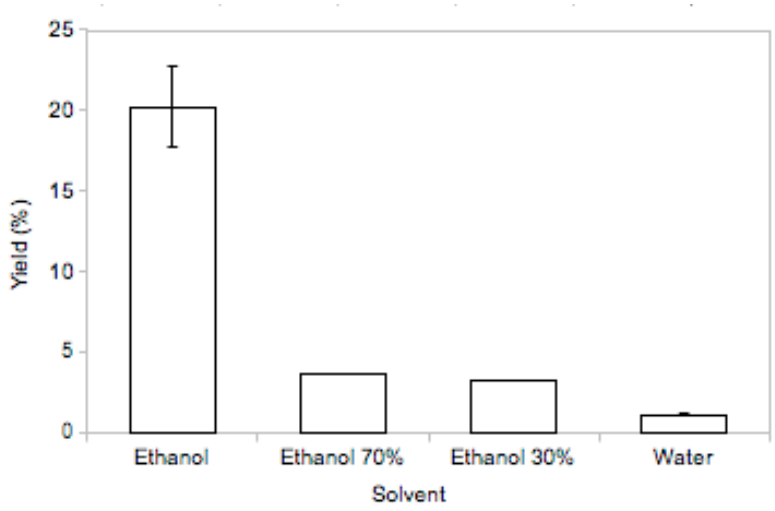

Figure 1. Yield percentage of *Phyllanthus acidus* fruit extract.

Results showed that the highest yield was given by the ethanol extract, while the lowest was given by the water extract. Extraction yields were different among the treatments because the bioactive components that are dissolved in those solvent also different. Wide ranges of chemical compounds easily dissolve in ethanol compared to water. Ethanol can dissolve less polar and polar compounds include alkaloids, steroids, saponines, flavonoids, quinones, and glicosides [8]. Natural products such as fruits contain those compounds. Water is commonly chosen due to its availability and price.

3.2 Phytochemical test

Secondary metabolites in *P. acidus* fruit were studied by qualitative phytochemical tests include flavonoids, alkaloids, tannins, saponines, steroids, terpenoids, phenolics, and glycosides (table 1). Results showed that all extracts contain flavonoids, alkaloids, phenolics, terpenoids, saponines, and glycosides. Although the components quantities were different in each solvent. Ethanolic extract contained more flavonoids, alkaloids, terpenoids, and saponines but less phenolics compare to the aqueous extract. All extract contained similar amount of glicosides and did not contain tannins and steroids.

Table 1. Phytochemical screening tests.

Phytochemical Tests	Extract			
	Ethanol	70% Ethanol	30% Ethanol	Water
Flavonoid	+++	+++	++	++
Tannin	-	-	-	-
Alkaloid	+++	+++	++	++
Phenolic	+	+	++	++
Terpenoid	+++	+++	++	++
Steroid	-	-	-	-
Saponine	+++	+++	+++	++
Glycoside	+	+	+	+

notes: (-) did not contain the components and (+) contains the components

Secondary metabolites are chemical components isolated from plant sources. These components act as protection for survival from predator and parasites. These compounds also can be acted as antioxidants [9]. Based on table 1, we suggest that flavonoid was the major constituents in our samples.

3.3 In vitro antioxidant activity

Among the samples, water extract of *P. acidus* exhibited high antioxidant activity with IC_{50} 26.06 µg/mL (figure 2). While ethanol extract did not give antioxidant activity. Lower value of IC_{50} means higher antioxidant activity, because we only need small amount of molecule to neutralize 50% of DPPH radicals in the solution.

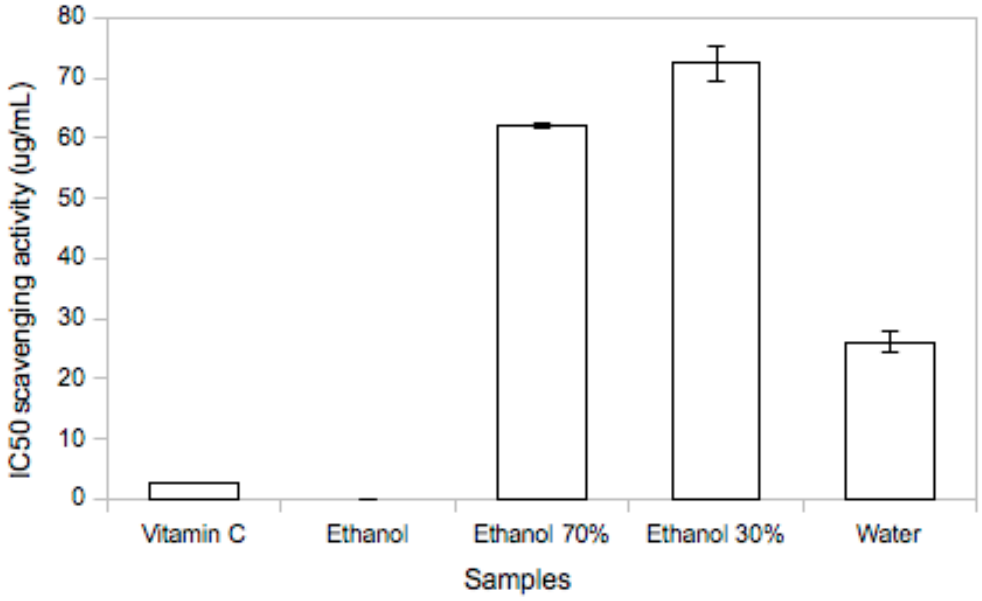

Figure 2. Antioxidant activity of *Phyllanthus acidus* fruit extract.

Natural products could be categorized as a strong antioxidant activity if is possess IC_{50} below 200 ⌈g/mL, while IC_{50} below 10 ⌈g/mL was considered as very strong antioxidant activity. Our results showed that ANOVA statistical test for all treatments different significantly (⟨<0.05). Hence, the solvents significantly gave effects in the number of bioactive chemical components extracted and furthermore give significant antioxidant activity differences.

3.4 Cytotoxicity test

LC_{50} values for BSLT ranged from 473.26 to 908.98 µg/ mL, with the water extract having the lowest value and therefore the most potent, and the ethanol extract having the highest value (figure 3). BSLT test is a simple bioassay test to study the acute toxicity of a chemical compound in *Artemia salina* Leach. The mechanism related with the ability of secondary metabolites in plant in poisoning the animal. 50% of lethal toxicity concentration (LC_{50}) was determined using probit analysis based on the number of mortality in *A. salina*. Juniarti [10] showed that potential bioactive crude extract posses LC_{50} value under 1000 ppm, while potential bioactive pure compounds have LC_{50} value below 30 ppm. *P.acidus* fruit extract has been reported to have antihyperlipidemic activity [2] and *P. acidus* leaves extract has activity as antiinflamatory, antinociceptive, and antioxidant have been reported by Chakraboty *et al*. [5].

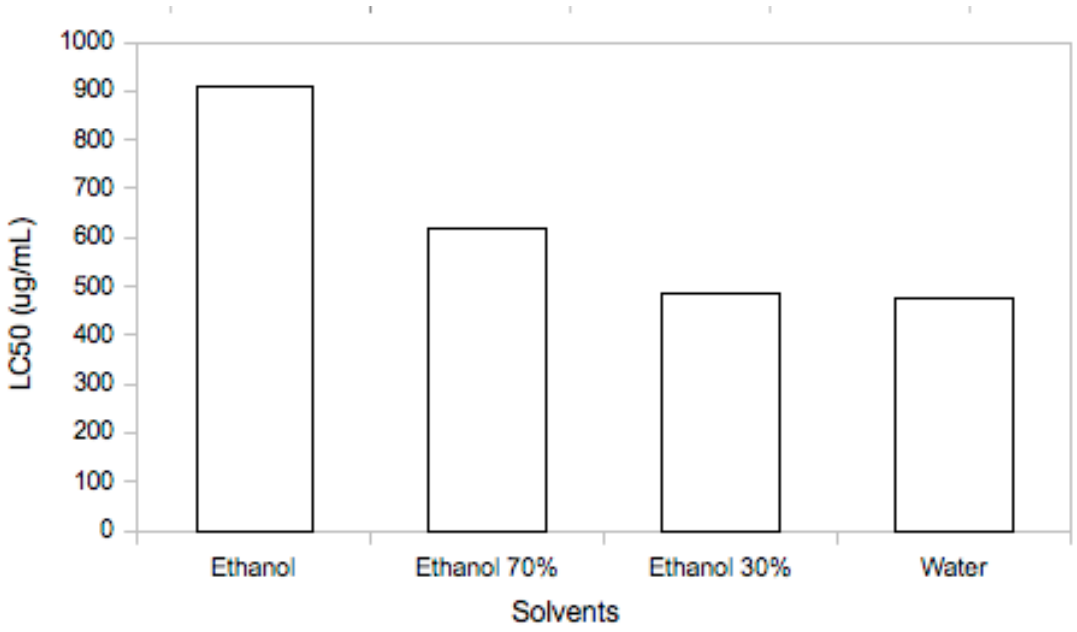

Figure 3. Cytotoxic activity of *Phyllanthus acidus* fruit extract.

4. Conclusion

This is the first report of antioxidant and cytotoxic activities of *Phyllanthus acidus* fruit ethanol and water extracts. We found that *P. acidus* water extract have the most potential as antioxidant and cytotoxic activities. Additional studies are needed to isolate and identify responsible compounds for those activities.

References

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