Determination of Phytochemical Constituent, Antioxidant Activity, Total Phenol and Total Flavonoid of Extract Ethanol Phyllanthus emblica Fruit

B Halim1,3, RA Syahputra2,*, I Adenin3, HP Lubis3, F Mendrofa4, S Lie5, SE Nugraha6

ABSTRACT

Introduction: Phyllanthus emblica (PE) is a plant that is regularly found in Indonesia, particularly on Sumatra Island. In India, it is known as Indian gooseberry and is frequently used in ayurvedic medicine. PE fruit is well-known for its high antioxidant activity and a variety of pharmacological properties. The purpose of this study was to ascertain the phytochemical composition, antioxidant activity, total phenol, and total flavonoid concentrations. Methods: The fruits were harvested in the Indonesian town of Padang Sidimpuan. Up to 700 g of dry PE fruit powder was dissolved in 96 percent ethanol and macerated for seven days, with periodic stirring daily. The solution was then filtered using Whatman paper no 1, and the filtered extract was evaporated under reduced pressure using a rotary evaporator until a crude extract/ethanol extract of PE (EEPE) was obtained, and the phytochemical constituents, antioxidant activity, total phenol, and flavonoid were analysed. Results: The result shows that EEPE contains some flavonoids such as quercetin, betaine, Trigonelline, Myricitrin, Myricetin, Leucine, and Kaempferol. EEPE as an antioxidant of 7.626 ± 0.41 µg/dL. It shows that the antioxidant activity of the ethanol extract of Phyllanthus emblica is strong ethanol extract of Phyllanthus emblica contains Total Flavonoid was 5.816 ± 2.81(mg QE/g extract) and total phenol was 274.590 ± 13.61(mg GAE/g extract). Conclusions: In summary, extract ethanol of Phyllanthus emblica contains flavono and have antioxidant activity and high total phenol and flavonoid levels.

Key words: Antioxidant, Total flavonoid, Total phenol, Phyllanthus emblica...

INTRODUCTION

Phyllanthus emblica (PE) is a plant that grows widely in Indonesia, particularly on Sumatra island. In India, it is known as Indian gooseberry and is frequently used in Ayurvedic medicine under the name Indian gooseberry. PE has long been used on a regular basis to promote hair development, relieve constipation, and alleviate fever and pain. Phyllanthus emblica is a member of the Euphorbiaceae family and is found across the subtropics and tropics, including China, India, Malaysia, and Thailand. The Phyllanthus emblica fruit is quite popular due to its high vitamin C and phenolic content. According to several reports, Phyllanthus emblica fruit possesses antioxidant, immunomodulatory, and anticancer properties. Analgesic, anti-pyretic, antidiabetic, and antimicrobial.1-4

All components of Phyllanthus emblica have been extensively employed in a variety of traditional remedies, including Indian Medicine (Ayurveda), Chinese Traditional Medicine, Tibetan Medicine, and Greek Arabic Medicine. Minority populations of southwest China use the root of Phyllanthus emblica to treat Eszema and the fruit to treat jaundice and diarrhoea. Additionally, it is utilised as an astringent and hemostatic in Nepal.7,8 The bark of Phyllanthus emblica has antioxidant activity and radical scavenging due to its polyphenol compounds.9,10 In China, the bark of Phyllanthus emblica is utilised for tannin extraction due to its high tannin content (between 21 and 33 percent). Numerous pharmacological investigations have found Phyllanthus emblica but have concentrated on the fruit, with other portions, such as the bark, receiving less attention. PE is an excellent source of metabolite chemicals, which include flavonoids, saponins, tannins, steroids, and glycosides. The flavonoid compounds contained in PE are kaemferol-3-O-a-L-(6″-methyl)-rhamnopyranoside, kaempferol-3-O-a-L-(6″-ethyl) rhamnopyranoside, and other compounds, such as Triacontanol, Triacontanic acid, β-Amyrin ketone, Betulinic acid, Daucosterol, Lupeol acetate, β-Amyrin-3-palmiate, Gallic acid, Betulnic acid, Ursolic acid, Oleanolic acid, Quercetin, Rutin, and Bisabolone. Also, PE fruit is rich in vitamin C, luteloin, and corilagin.11 This study aim to determine the antioxidant activity, total phenol, and total flavonoid of Phyllanthus emblica ethanol extract.

MATERIALS AND METHODS

Plant collection

Fruits were obtained from Padang Sidimpuan, North Sumatra, Indonesia (01o 08' 07''- 01o 28' 19'' North Latitude and 99o 13' 53''- 99o 21' 31'' East Longitude). After washed and dried, the fruits were crushed until obtaining dry fruit powder.

Extract ethanol Phyllanthus emblica preparation

700 g of dry PE fruit powder was dissolved in 96 percent ethanol and macerated for seven days, with periodic steering on a daily basis. After filtering...
the solution using Whatman paper no 1, the filtrate was evaporated under reduced pressure in a rotary evaporator till crude extract/ethanol extract of PE (EEPE) was produced. Then, phytochemical screening was undertaken (alkaloids, flavonoids, tannins, saponins, glycosides, steroids/triterpenoids).

**Phytochemical constituent analysis by LC-HRMS**

The phytochemical analysis of *Phyllanthus emblica* ethanol extract was performed using the TSQ Exactive (Thermo) gradient technique (LSIH, Brawijaya University) with mobile phase A (0.1 percent formic acid in water) and phase B. (0.1 percent formic acid in acetonitrile). The 501mm1.9m Hypersil GOLD Q column was analysed for 70 minutes at a flow rate of 40L/min. CompoundDiscoverersoftwarewas used in conjunction with mzCloud to analysethe data.

**DPPH scavenging activity**

The DPPH scavenging activity was determined using a slightly modified Blois technique. We dissolved up to 25 mg EEPE in 25 mL methanol and sonicated for 30 minutes (40 °C). It was then centrifuged at 1000 rpm for 10 minutes and diluted to get 6.25 g/mL, 12.5 g/mL, 25 g/mL, 50 g/mL, and 100 g/mL concentrations. Up to 20 mg DPPH was dissolved in 100 mL methanol (200 g/mL) and sonicated for 30 minutes at 40 °C, followed by centrifugation at 100 rpm for 10 minutes and dilution to reach a control concentration of 40 g/mL. The extract solution was combined with DPPH, vortexed, and left at a temperature of 270°C for 30 minutes. It was then subjected to 517 nm using a spectrophotometer. The formula is as follows:

\[
\text{DPPH scavenging activity (IC50) = (Absorbance Control - Absorbance Sample)/Absorbance
}\]

**Total flavonoid content (TFC)**

A total of 10.5 mg ethanol extract of PE was diluted in methanol to a volume of 10 mL, pipetted 0.5 mL solution, and then added 1.5 mL methanol, 0.1 mL 10% aluminum chloride solution, 0.1 mL 1 M sodium acetate solution, and 2.8 mL pure water. At a wavelength of 436 nm, measurements were taken five times. The concentration of flavonoids was determined using the substitution method in the linear regression equation and expressed as the equivalent milligrams of quercetin in 1 gramme of extract.

**RESULTS AND DISCUSSION**

**Phytochemical analysis of ethanol extract of PE**

Determine the composition of ethanol extract of PE used LC-HRMS to analyse its phytochemical constituents. Table 1 summarises the findings.

The results showed that extract ethanol of PE contains some flavonoids such as quercetine, betaine, Trigonelline, Myricitrin, Myricetin, Leucine, and Kaempferol.

**Total flavonoid dan phenol content**

Total flavonoids from EEPE used gallic acid as a comparison. Total flavonoids contained in the extract and total phenols from EEPE used quercetin as a comparison. The regression equation for determining phenolic content is \( y = 0.00125X + 0.0252 \), while the regression equation for total flavonoids is \( y = 0.03689X + 0.0013 \). The results of total flavonoids and total phenol can be seen in Table 3.

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**Table 1: DPPH scavenging activity of PE.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Formula</th>
<th>Molecular Weight</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Quercetine</td>
<td>C15H10O7</td>
<td>302.04256</td>
<td>8.125</td>
</tr>
<tr>
<td>2.</td>
<td>Betaine</td>
<td>C5H11NO2</td>
<td>117.0792</td>
<td>1.086</td>
</tr>
<tr>
<td>3.</td>
<td>Trigonelline</td>
<td>C7H7NO2</td>
<td>137.04779</td>
<td>0.979</td>
</tr>
<tr>
<td>4.</td>
<td>Stearamide</td>
<td>C18H37NO</td>
<td>283.28737</td>
<td>24.585</td>
</tr>
<tr>
<td>5.</td>
<td>Ellagic acid</td>
<td>C14H6O8</td>
<td>302.00627</td>
<td>7.248</td>
</tr>
<tr>
<td>6.</td>
<td>Myricitrin</td>
<td>C21H20O12</td>
<td>464.09595</td>
<td>7.283</td>
</tr>
<tr>
<td>7.</td>
<td>Myricetin</td>
<td>C15H10O8</td>
<td>318.03789</td>
<td>7.277</td>
</tr>
<tr>
<td>8.</td>
<td>Leucine</td>
<td>C5H13NO2</td>
<td>131.09477</td>
<td>0.956</td>
</tr>
<tr>
<td>10.</td>
<td>α-Linolenic acid</td>
<td>C18H30O2</td>
<td>278.28269</td>
<td>20.586</td>
</tr>
</tbody>
</table>

**Table 2: DPPH scavenging activity of PE.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Extract</th>
<th>IC50 (µg/dL) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethanol extract of PE</td>
<td>7.626 ± 0.41</td>
</tr>
</tbody>
</table>

**Table 3: Total flavonoid and phenol.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Extract</th>
<th>Total Flvnonoid (mg QE/g extract)</th>
<th>Tota Phenol (mg GAE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethanol extract of PE</td>
<td>5.816 ± 2.81</td>
<td>274.590 ± 13.61</td>
</tr>
</tbody>
</table>
The table 3 above shows the ability of ethanol extract of *Phyllanthus emblica* contains Total Flavonoid was 5.816 ± 2.81(mg QE/g extract) and total phenol was 274.590 ± 13.61(mg GAE/g extract).

*Phyllanthus emblica* (PE) or Indian gooseberry has been widely used as traditional and Ayurvedic medicine in India. PE is commonly spread in Southeast Asia, including Malaysia and Indonesia. A comprehensive toxicity evaluation is needed to ensure the safe use of PE. The active compounds found in PE are apigenin-7-O-((6'-butyl-β-glucopyranoside), gallic acid, and luteolin-4’-O-neohesperidoside. This compound has an antioxidant role; in this study, the scavenging ability of EEPE has an IC50 value of 7.626 ± 0.41 µg/dL (Table 2). Another study also revealed that luteolin has several cardioprotective mechanisms by means of anti-calciump overload, other luteolin functions can also reduce radical compounds (O-, H2O2, and OH-), ability of EEPE has an IC50 value of 7.626 ± 0.41 µg/dL (Table 2). Moreover, Luteolin has LD50 higher than 5000 mg/kgBW, while Gallic acid has LD50 more than 2000 mg/kgBW.

CONCLUSION

In summary, extract ethanol of *Phyllanthus emblica* contains flavonoid and have antioxidant activity and high total phenol and flavonoid levels. For further investigation, *Phyllanthus emblica* extract can be tested into several pharmacological activities by in vitro and in vivo methods.

REFERENCES

GRAPHICAL ABSTRACT

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